CUK-H01338-27-P024288

January 1987
volume 156, *number* 1

Acc.
16.12.87

American Journal of OBSTETRICS AND GYNECOLOGY

Copyright © 1987 by The C. V. Mosby Company

27

Editor in Chief
JOHN I. BREWER

Editors
FREDERICK P. ZUSPAN • E. J. QUILLIGAN

Associate Editor
ALBERT B. GERBIE

Official Publication

AMERICAN GYNECOLOGICAL AND OBSTETRICAL SOCIETY
CENTRAL ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
SOCIETY OF OBSTETRICIANS AND GYNAECOLOGISTS OF CANADA
SOUTH ATLANTIC ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
PACIFIC COAST OBSTETRICAL AND GYNECOLOGICAL SOCIETY
AMERICAN BOARD OF OBSTETRICS AND GYNECOLOGY
SOCIETY FOR GYNECOLOGIC INVESTIGATION
SOCIETY OF PERINATAL OBSTETRICIANS

Published by

THE C. V. MOSBY COMPANY
St. Louis, Missouri 63146

ISSN 0002-9378

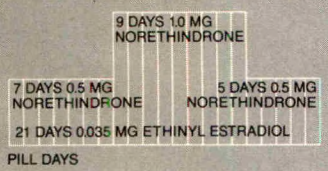


UNIQUE

Full-cycle endometrial support, with no late-cycle progestin increase.



The unique TRI-NORINYL phasing achieves BTB control equivalent to a 1/35 formulation, but with 29% less progestin per cycle. This is, in fact, the greatest progestin reduction of any norethindrone-based multiphasic. TRI-NORINYL lowers progestin where requirements for endometrial support are reduced—with no greater reported BTB than a 1/35 formulation.*



SUPPLIED IN THE EXCLUSIVE SYNTEX WALLETTE™ PILL DISPENSER



Preferred by patients 3 to 1 over other available packages for convenience, discreetness and compliance.**

A.J.O.G.
1. 156-157.
1-3, 2
1987

P24, 2-88

THE NATURE OF TRI-NORINYL®

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

*1/35 formulations contain 1.0 mg norethindrone with 0.035 mg ethinyl estradiol. Data available from Syntex Laboratories, Inc.

**Based on a nationwide, independently conducted survey of prospective oral contraceptive patients. Data available from Syntex Laboratories, Inc.

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see brief summary of full prescribing information on next page.

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

©1986 Syntex Laboratories, Inc.

THE NATURE OF TRI-NORINYL®

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

TRI-NORINYL® 21-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)

TRI-NORINYL® 28-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg; 7 inert tablets)

ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications Prevention of pregnancy. *DOSE-RELATED RISK OF THROMBOEMBOLISM.* Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism, CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OC. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested the persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34–5/100,000; ages 35-44–33/100,000; ages 45-49–140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34–1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over–1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and for MI is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS–RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40+
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A–Use associated with very high risk.
B–Use associated with high risk.
C–Use associated with moderate risk.
D–Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden, or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to the registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer, and other studies suggested an increased risk of breast cancer with long-term OC use before age 25. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared to nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. Two other studies suggested an increasing incidence of cervical neoplasms with longer duration of OC use. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OCs with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones–estrogenic and progestational agents–during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase the risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in the results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. One study suggests *Chlamydia trachomatis* infection, the leading cause of nongonococcal P.I.D., is enhanced by OCs. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failure. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions** *GENERAL* 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding from interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, antimigraine preparations, antihistamines, and griseofulvin. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, theophylline, and beta blockers. Toxic liver effects have been reported with a combination of an OC and cyclosporin A. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Warnings. **Adverse Reactions** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps, colitis), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, changes in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution of lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chilblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, vaginitis, porphyria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome), malignant hypertension, acute renal failure. **Information for the Patient** (See Patient Package Insert).

Rev. 6/86

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

A Standard in Progesterone Therapy

Provera® 10 MG TABLETS
(medroxyprogesterone acetate)

WARNING

THE USE OF PROGESTATIONAL AGENTS DURING THE FIRST FOUR MONTHS OF PREGNANCY IS NOT RECOMMENDED.

Progestational agents have been used beginning with the first trimester of pregnancy in an attempt to prevent habitual abortion or treat threatened abortion. There is no adequate evidence that such use is effective and there is evidence of potential harm to the fetus when such drugs are given during the first four months of pregnancy. Furthermore, in the vast majority of women, the cause of abortion is a defective ovum, which progestational agents could not be expected to influence. In addition, the use of progestational agents, with their uterine-relaxant properties, in patients with fertilized defective ova may cause a delay in spontaneous abortion. Therefore, the use of such drugs during the first four months of pregnancy is not recommended.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies including congenital heart defects and limb reduction defects. One study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000.

If the patient is exposed to PROVERA Tablets (medroxyprogesterone acetate) during the first four months of pregnancy or if she becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus.

INDICATIONS: Secondary amenorrhea; abnormal uterine bleeding due to hormonal imbalance in the absence of organic pathology such as fibroids or uterine cancer.

CONTRAINDICATIONS: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy or patients with a past history of these conditions. Liver dysfunction or disease. Known or suspected malignancy of breast or genital organs. Undiagnosed vaginal bleeding. Missed abortion. Known sensitivity to medroxyprogesterone acetate. As a diagnostic test to pregnancy.

WARNINGS: 1. Immediately discontinue administration should any of the following thrombotic disorders occur or be suspected: thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis. **2.** Beagle dogs treated with medroxyprogesterone acetate developed mammary nodules some of which were malignant. Although nodules occasionally appeared in control animals, they were intermittent in nature; whereas the nodules in the drug treated animals were larger, more numerous, persistent, and there were some breast malignancies with metastases. Their significance with respect to humans has not been established. **3.** Discontinue medication pending examination if there is sudden partial or complete loss of vision, onset of proptosis, diplopia, or migraine. If papilledema or retinal vascular lesions occur, withdraw medication. **4.** Detectable amounts of progestin have been identified in the milk of mothers receiving the drug. The effect of this on the nursing infant has not been determined. **5.** Usage in pregnancy is not recommended (see Warning box). **6.** Three major studies in Great Britain and one in this country have shown a statistically significant association between thrombophlebitis, pulmonary embolism, cerebral thrombosis and embolism and the use of oral contraceptives. It has been estimated that users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study indicated that the risk did not persist after discontinuation, and it was not enhanced by long-continued administration.

PRECAUTIONS: A pretreatment physical exam should include special reference to breast and pelvic organs and a Papanicolaou smear. This drug may cause fluid retention; therefore, carefully observe patients with conditions influenced by fluid retention such as epilepsy, migraine, asthma, and cardiac or renal dysfunction. In irregular bleeding per vaginum, bear in mind nonfunctional causes and perform adequate diagnostic measures. Advise pathologist of therapy when submitting relevant specimens. Carefully observe patients with history of psychic depression and discontinue drug if serious depression recurs. Any possible influence of prolonged therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study. Decreased glucose tolerance has been observed in a small percentage of patients on estrogen-progestin combinations; therefore, carefully observe diabetic patients receiving progestin therapy. Age constitutes no absolute limiting factor, although onset of climacteric may be masked. Because of the occasional occurrence of thrombotic disorders (thrombophlebitis, pulmonary embolism, retinal thrombosis, and cerebrovascular disorders) in patients taking estrogen-progestin combinations and since the mechanism is obscure, the physician should be alert to the earliest manifestation of these disorders. (See Patient Information for complete prescribing information.)

ADVERSE REACTIONS: Pregnancy: (see Warning box); **Breast:** rare reports of breast tenderness or galactorrhea; **Skin:** sensitivity reactions including pruritus, urticaria, edema and generalized rash; acne, alopecia and hirsutism in a few patients; **Thromboembolic Phenomena** including thrombophlebitis and pulmonary embolism.

The following adverse reactions have been observed in women taking progestins including medroxyprogesterone acetate: breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; edema; change in weight; changes in cervical erosion and secretions; cholestatic jaundice; rash (allergic) with and without pruritus; mental depression; anaphylaxis and anaphylactoid reactions; pyrexia; insomnia; nausea and somnolence.

A statistically significant association has been demonstrated between use of estrogen-progestin combination drugs and the serious adverse reactions of thrombophlebitis, pulmonary embolism and cerebral thrombosis and embolism. Therefore, patients on progestin therapy should be carefully observed.

Although available evidence is suggestive, a relationship has been neither confirmed nor refuted for the association of the serious adverse reaction of neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been observed in patients receiving estrogen-progestin combination drugs: rise in blood pressure in susceptible individuals; premenstrual-like syndrome; changes in libido; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; fatigue; backache; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; and itching.Therefore, observe patients on progestin therapy carefully.

The following laboratory results may be altered by the use of estrogen-progestin combination drugs: increased sulfobromophthalein retention and other hepatic function tests; coagulation tests (increase in prothrombin factors VII, VIII, IX and X); metyrapone test; pregnanediol determination; thyroid function tests (increase in PBI, and butanol extractable protein bound iodine and decrease in T^3 uptake values).

Caution: Federal law prohibits dispensing without prescription.

MED B-13-S

Upjohn

A Century of Caring
1886·1986

March 1986

J-6144

© 1986 The Upjohn Company

The Upjohn Company, Kalamazoo, MI 49001

A Standard



Provera® 10 MG TABLETS
(medroxyprogesterone acetate)

A Standard in Progesterone Therapy

Please see adjacent page for brief summary of prescribing information.

MOSBY

American Journal of Obstetrics and Gynecology

Founded in 1920

Copyright © 1987 by The C. V. Mosby Company

Contents

January
1987

CLINICAL SECTION

Clinical Opinion

Screening for postmenopausal osteoporosis 1
Margaret R. Davis, M.D.
Washington, D. C.

Breech delivery: Why the dilemma? 6
Stephen A. Myers, D.O., and Norbert Gleicher, M.D.
Chicago, Illinois

Clinical Articles

Immunoproteins in the endometrium: Clinical correlates of the presence of complement fractions C3 and C4 11
Delphine Bartosik, M.D., Ivan Damjanov, M.D., Ph.D., Richard R. Viscarello, M.D., and Judith A. Riley, M.D.
Philadelphia, Pennsylvania

(Contents continued on page 6A)

Vol. 156, No. 1, January 1987. The American Journal of Obstetrics and Gynecology is published monthly by The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146. POSTMASTER: Send address changes to The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

1987 Annual subscription rates	U.S.A.	International (surface mail) All regions	International (airmail)* Canada & Mexico	Region 1	Region 2	Region 3
Institutions†	$102.00	$121.00	$142.55	$153.60	$183.85	$214.10
Individuals‡	$ 59.50	$ 78.50	$100.05	$111.10	$141.35	$171.60
Students, residents‡	$ 40.00	$ 59.00	$ 80.55	$ 91.60	$121.85	$152.10

Single copies are $6.50. Remittances should be made by check, draft, or post office or express money order, in U.S. funds drawn through a U.S. bank, payable to this JOURNAL. Claims for missing issues will be serviced only within six months of cover date. Single copy prices will be charged on missing issue claims older than six months from cover date.

*Airmail breakdown—Domestic: First-class and Priority rates for the U.S. and possessions are available upon request. *Region 1:* Central America, islands, and mainland colonies of European countries in The Americas. *Region 2:* South America, Europe, Egypt, Africa (bordering the Mediterranean). *Region 3:* Asia, Australasia, Africa (other than Mediterranean), Middle East, Far East, The Pacific, U.S.S.R. (and constituent Republics).

†Institutional (multiple-reader) subscriptions are available to public and private libraries, schools, hospitals, and clinics; city, county, state, provincial, and national government bureaus and departments; and all commercial and private institutions and organizations.

‡Individual subscriptions and all student-rate subscriptions must be in the names of, billed to, and paid by individuals. All student-rate requests must indicate training status and name of institution.

Subscriptions may begin at any time.

Second-class postage paid at St. Louis, Missouri, and additional mailing offices. Printed in the U.S.A. Copyright © 1987 by The C. V. Mosby Company.

Contents continued from page 5A

Labial fusion in prepubescent girls: A marker for sexual abuse? 16
Carol D. Berkowitz, M.D., Sandra L. Elvik, R.N., B.S.N., C.P.N.P., and
Mary K. Logan, R.N., B.S.N., P.H.N.
Torrance, California

Postmenopausal smoking, estrogen replacement therapy, and the risk of endometrial cancer 20
Adele L. Franks, M.D., Juliette S. Kendrick, M.D., Carl W. Tyler, Jr., M.D., and
The Cancer and Steroid Hormone Study Group
Atlanta, Georgia

The efficacy of intrapartum electronic fetal monitoring 24
Stephen B. Thacker, M.D.
Atlanta, Georgia

A high prevalence of chlamydial cervicitis in postmenopausal women 31
Tetsuo Nagashima, M.D.
Yokosuka, Japan

Alcohol teratogenicity in the human: A detailed assessment of specificity, critical period, and threshold 33
Claire B. Ernhart, Ph.D., Robert J. Sokol, M.D., Susan Martier, M.S.S.A.,
Pamela Moron, B.S., Debra Nadler, B.S., Joel A. Ager, Ph.D.,
and Abraham Wolf, Ph.D.
Cleveland, Ohio, and Detroit, Michigan

Abruptio placentae: Clinical management in nonacute cases 40
John S. Sholl, M.D.
Evanston, Illinois

Randomized management of the second nonvertex twin: Vaginal delivery or cesarean section 52
Jaron Rabinovici, M.D., Gad Barkai, M.D., Brian Reichman, M.B., Ch.B.,
David M. Serr, M.B., Ch.B., and Shlomo Mashiach, M.D.
Tel-Hashomer and Tel Aviv, Israel

Predictors of vaginal delivery in patients with a previous cesarean section, who require oxytocin 57
Richard K. Silver, M.D., and Ronald S. Gibbs, M.D.
San Antonio, Texas

The effect of percutaneous estradiol and natural progesterone on postmenopausal bone loss 61
Bente J. Riis, M.D., Karsten Thomsen, M.D., Verner Strøm, M.D., and
Claus Christiansen, M.D.
Glostrup, Denmark

(Contents continued on page 9A)

Mycelex® Twin Pack
(clotrimazole)

One-day therapy

Mycelex®-G 500 mg
(clotrimazole) Vaginal Tablet

The shortest regimen for vulvovaginal candidiasis

In the case of severe vulvovaginitis due to candidiasis, longer antimycotic therapy is recommended.

Please see following page for brief summary of prescribing information.

Seven-day therapy

Mycelex® 1%
(clotrimazole) Cream

For prompt relief of cutaneous symptoms



Mycelex® Twin Pack

Miles Pharmaceuticals
Division of Miles Laboratories, Inc.
West Haven, Connecticut 06516 USA

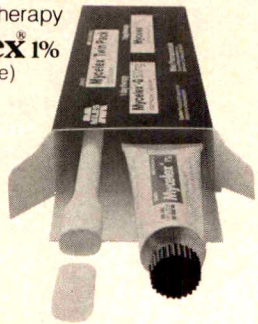
© 1986 Miles Pharmaceuticals Division of Miles Laboratories, Inc.

MILES
Miles Pharmaceuticals

Mycelex® Twin Pack
(clotrimazole)

One-day therapy
Mycelex®-G 500mg
(clotrimazole) Vaginal Tablet

Seven-day therapy
Mycelex® 1%
(clotrimazole)
Cream



Indications
MYCELEX®-G 500 mg Vaginal Tablets are indicated for the local treatment of vulvovaginal candidiasis when one-day therapy is felt warranted. In the case of severe vulvovaginitis due to candidiasis, longer antimycotic therapy is recommended.
MYCELEX® CREAM is indicated for the topical treatment of dermal infections due to *Candida albicans*.

Contraindications
MYCELEX-G 500 mg Vaginal Tablets and MYCELEX CREAM are contraindicated in women who have shown hypersensitivity to any of their components.

Warnings
MYCELEX-G 500 mg Vaginal Tablets: none. MYCELEX CREAM is not for ophthalmic use.

Precautions
MYCELEX-G 500 mg Vaginal Tablets: If there is a lack of response to the Vaginal Tablets, appropriate microbiological studies should be repeated to confirm the diagnosis and rule out other pathogens before instituting another course of antimycotic therapy.
MYCELEX CREAM: If irritation or sensitivity develops with the use of Mycelex Cream, treatment should be discontinued and appropriate therapy instituted.

Carcinogenesis
MYCELEX-G 500 mg Vaginal Tablets: No long-term studies in animals have been performed to evaluate the carcinogenic potential of 500 mg Vaginal Tablets intravaginally. A long-term study in rats (Wistar strains) where clotrimazole was administered orally provided no indication of carcinogenicity.

Usage in Pregnancy: Pregnancy Category B:
MYCELEX-G 500 mg Vaginal Tablets: The disposition of ^{14}C-clotrimazole has been studied in humans and animals. Clotrimazole is poorly absorbed following intravaginal administration to humans, whereas it is rather well absorbed after oral administration.
In clinical trials, use of vaginally applied clotrimazole in pregnant women in their second and third trimesters has not been associated with ill effects. There are, however, no adequate and well-controlled studies in pregnant women during the first trimester of pregnancy.
Because animal reproduction studies are not always predictive of human response, this drug should be used only if clearly indicated during the first trimester of pregnancy.
MYCELEX CREAM: In the first trimester of pregnancy, Mycelex Cream should be used only when considered essential to the welfare of the patient.

Adverse Reactions
MYCELEX-G 500 mg Vaginal Tablets: Of 297 patients in double-blind studies with the 500 mg vaginal tablet, 3 of 149 patients treated with active drug and 3 of 148 patients treated with placebo reported complaints during therapy that were possibly drug related. In the active drug group, vomiting occurred in one patient, vaginal soreness with coitus in another, and complaints of vaginal irritation, itching, burning and dyspareunia in the third patient. In the placebo group, clitoral irritation occurred in one patient and dysuria, described as remotely related to drug, in the other. A third patient in the placebo group developed bacterial vaginitis which the investigator classed as possibly related to drug.
MYCELEX CREAM: The following reactions have been reported in connection with the use of this cream: erythema, stinging, blistering, peeling, edema, pruritus, urticaria, and general irritation of the skin.

Issued, July 1986

Miles Pharmaceuticals
Division of Miles Laboratories, Inc.
West Haven, Connecticut 06516 USA

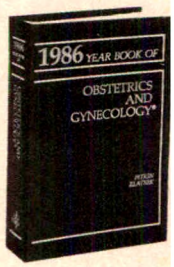
MILES
Miles Pharmaceuticals

THE 1986 YEAR BOOK OF

OBSTETRICS AND GYNECOLOGY®

Edited by: Roy M. Pitkin, M.D., and Frank J. Zlatnik, M.D.

THE YEAR BOOK CONCEPT:



- Clinically oriented
- Condensations of the most vital journal articles worldwide chosen with your needs in mind—studies, discoveries, and developments that will affect your practice today. . .and tomorrow
- Pointed editorial comments on the articles—assessing their validity, relating them to other studies, and spotting new trends in the field
- A handsome, compact volume complete with illustrations, indexes, and references—a virtual storehouse of information enriching your professional library

1986 539 pp./229 illus. $42.95*

FREE ON 30-DAY APPROVAL!

Please rush me the following title for a free, trial examination:
____ **The 1986 Year Book of Obstetrics and Gynecology®**
(5-86) $42.95*
Dial toll-free 800/621-9262.
In Illinois, call collect (312) 726-9746.

NAME _____
ADDRESS _____
CITY _____
STATE _____ ZIP _____
PHONE _____
CIRCLE ONE: Visa/Master Card/AMX # _____
SIGNATURE _____

YEAR BOOK MEDICAL PUBLISHERS
35 EAST WACKER DRIVE
CHICAGO, ILLINOIS 60601

*This book is published annually. For the convenience of its customers, Year Book enters each purchaser as a subscriber to future volumes and sends annual announcements of each volume approximately two months before publication. The new volume will be shipped upon publication unless you complete and return the cancellation notice attached to the announcement and it is received by Year Book within the time indicated (approximately 20 days after your receipt of the announcement). You may cancel your subscription at any time. The new volume may be examined on approval for 30 days, may be returned for full credit, and if returned Year Book will then remove your name as a subscriber. Return postage is guaranteed by Year Book to the Postal Service.

Prices quoted are in U.S. dollars. Canadian orders will be billed in Canadian funds at the approximate current exchange rate. A small additional charge will be made for postage and handling. Illinois and Tennessee residents will be billed appropriate sales tax. All prices quoted subject to change. Title marked (†) is forthcoming; please do not send cash for this book.

ZZMISC

Contents continued from page 6A

Long-term effects of percutaneous estrogens and oral progesterone on serum lipoproteins in postmenopausal women 66
Jytte Jensen, M.D., Bente J. Riis, M.D., Verner Strøm, M.D., Lisbeth Nilas, M.D., and Claus Christiansen, M.D.
Glostrup, Denmark

Use of the urinary director appliance for management of voiding problems after radical vulvectomy 72
Karen L. Ilika, M.D., and Patrick Duff, M.D.
Tacoma, Washington

Liposarcoma presenting on the vulva 73
John J. Brooks, M.D., and Virginia A. LiVolsi, M.D.
Philadelphia, Pennsylvania

Serum CA-125 antigen levels increase during menses 75
Donald E. Pittaway, M.D., Ph.D., and Jamil A. Fayez, M.D.
Winston-Salem, North Carolina

Advanced ultrasonic placental maturation in twin pregnancies 76
G. Ohel, M.D., M. Granat, M.D., D. Zeevi, M.D., A. Golan, M.D., Sh. Wexler, M.D., M. P. David, M.D., and J. G. Schenker, M.D.
Jerusalem and Tel Aviv, Israel

Antenatal sonography of fetal malformations associated with drugs and chemicals: A guide 79
Gideon Koren, M.D., A.B.M.T., Michelle Brille Edwards, M.D., and Murray Miskin, M.D.
Toronto, Ontario, Canada

Fryns syndrome: A predictable, lethal pattern of multiple congenital anomalies 86
Arnon Samueloff, M.D., Daniel Navot, M.D., Arie Birkenfeld, M.D., and Joseph G. Schenker, M.D.
Jerusalem, Israel

Unruptured pregnancy in a heterotopic fallopian tube: Evidence for transperitoneal sperm migration 88
Charles Brown, M.D., William E. LaVigne, M.D., and Santiago L. Padilla, M.D.
Augusta, Georgia

Laparoscopy—A diagnostic aid in cases of suspected appendicitis 90
Nick M. Spirtos, M.D., Scott M. Eisenkop, M.D., Tanya W. Spirtos, M.D., Raymond I. Poliakin, M.D., and Lester T. Hibbard, M.D.
Los Angeles, California

(Contents continued on page 12A)

The simplest way to

There are still many who perform a CBC or hematocrit as soon as anemia is suspected, even though it is in fact the Hb value that defines anemia.

There is a simpler way.

Determine the Hb level directly. If it lies below the reference standard, it is reasonable to suspect anemia. A detailed patient history will then disclose whether it is caused by a deficiency in iron or loss of blood.

But...

The Hb level varies from person to person. It thus makes sense to monitor each individual patient's Hb level routinely to identify early signs of anemia.

For this you would require a test procedure that could be performed in-office with minimal risk of functional and human error.

This is where the HemoCue Hb test comes in.

The HemoCue system is designed to eliminate possible sources of error. It is so simple to handle that anyone can perform accurate Hb determinations in 60 seconds or less.

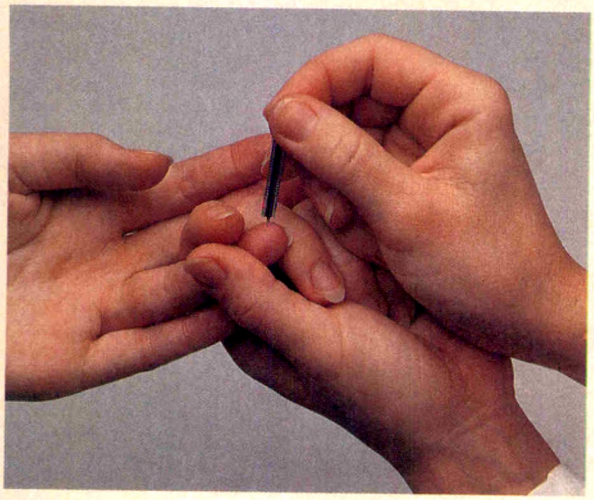
Going the long way around through CBC, or via hematocrit, you can get there quicker and safer with HemoCue. And have the test result before the patient leaves your office!

For more information on this new, economical test, call 800-323-1674 (CA 714-859-7779).

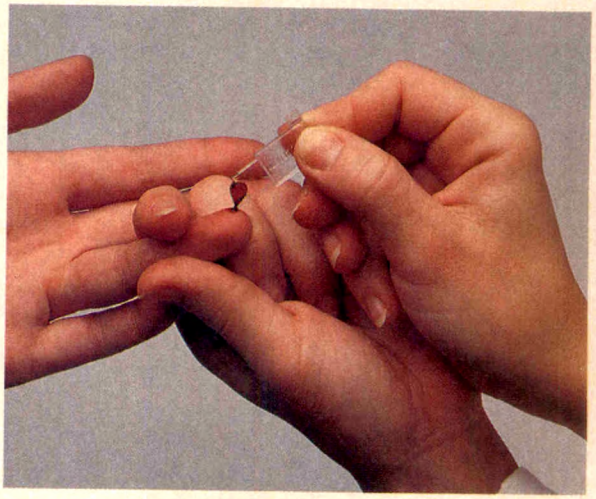
Sales and service outside the U.S.:
Denmark: Ercopharm A/S. Tel. (02) 89 08 89. **Finland:** Oy Tamro Ab. Tel. 90-544 011. **Norway:** A/S Saba Medical. Tel. (02) 64 47 30. **Iceland:** G Olafsson h/f. Tel. 01-8
Greece: Pharmaserve SACI. Tel. 01-6726 380. **Ireland:** Accu-Science (Ireland) Ltd. Tel. 045-603 91. **Italy:** Labanalysis srl. Tel. (06) 45 03 918. **The Netherlands:**
UCB Farma Nederland BV. Tel. 076-87 92 22. **Switzerland:** Baumann-Medical. Tel. 01-932 30 92. **France:** TLM Laboratoire SARL. Tel. (1) 39 65 69 09.
Great Britain: Clandon Scientific Ltd. Tel. (0252) 5147 11/5. **Australia & New Zealand:** Medipac Pty Ltd. Tel. 043-28 20 26. **Pakistan:** S. Ejazuddin & Co.
Tel. (Karachi) 22 44 20. **Singapore:** Transmedic Pte Ltd. Tel. 274 66 33. **Malaysia:** Translab (M) Sdn Bhd. Tel. 03-78 10 44. **Indonesia:** Berca Indonesia Pt. Tel. 021-34
Hongkong & Macao: Science International. Tel. 05-43 74 42. **Taiwan:** Transmedic Co Ltd. Tel. 02-981 5343. **South Korea:** Poong Nam Corp. Tel. (02) 557-6947.

diagnose anemia.

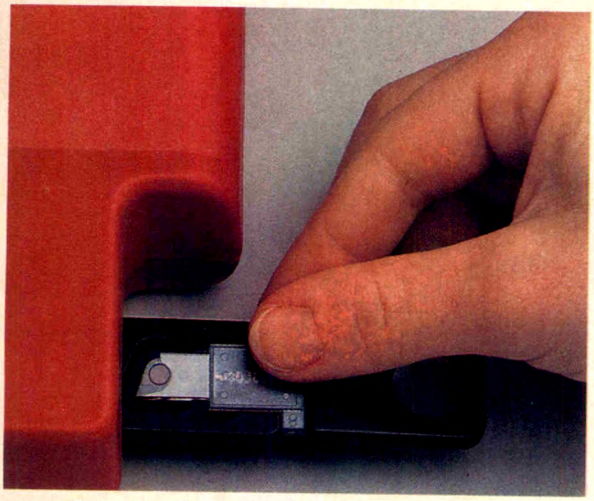
The Hemocue procedure.



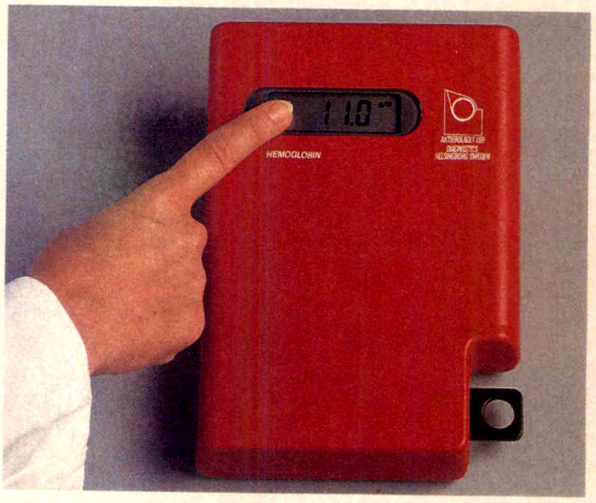
1. *Obtain a blood sample by finger stick.*



2. *The HemoCue microcuvette draws an exact volume of blood.*



3. *Insert the microcuvette into the HemoCue photometer.*



4. *Result displayed automatically within 15–45 seconds.*

HemoCue®

Developed and manufactured by Leo Diagnostics AB, S-251 09 Helsingborg, Sweden.
Marketing and technical service in the USA exclusively through
Medical Equipment Designs, Inc. 23521 Ridge Route Dr. Suite A, Laguna Hills, CA 92653.

Contents continued from page 9A

Proliferation of group B streptococci in human amniotic fluid in vitro 95
Iffath A. Abbasi, Lieutenant Commander, MC, USNR, Val G. Hemming, Colonel, MC, USAF, Gary S. Eglinton, Colonel, MC, USAF, and Timothy R. B. Johnson, Major, MC, USAF
Bethesda, Maryland

Urinary diagnostic indices in preeclampsia-associated oliguria: Correlation with invasive hemodynamic monitoring 100
Wesley Lee, M.D., Bernard Gonik, M.D., and David B. Cotton, M.D.
Houston, Texas

Adjunctive magnesium sulfate infusion does not alter metabolic changes associated with ritodrine tocolysis 103
James E. Ferguson II, M.D., R. Harold Holbrook, Jr., M.D., David K. Stevenson, M.D., Paul A. Hensleigh, M.D., Ph.D., and Daniel Kredentser, M.D.
Stanford and San Jose, California

Radioimmunoscintigraphic detection of occult gestational choriocarcinoma 108
Richard L. Wahl, M.D., M. B. Khazaeli, Ph.D., Albert F. LoBuglio, M.D., R. A. Patillo, M.D., Michael J. Tuscan, C.N.M.T., and William H. Beierwaltes, M.D.
Ann Arbor, Michigan, Birmingham, Alabama, and Milwaukee, Wisconsin

Corticosteroid levels in human fetal blood at midgestation and at term 112
A. López Bernal, E.O.G., D.Phil., and Ian Z. MacKenzie, M.D.
Oxford, England

Anticardiolipin antibodies and complement in ninety-nine women with habitual abortion 114
A. Margareta Unander, M.D., Ph.D., Renée Norberg, M.D., Ph.D., Lennart Hahn, M.D., Ph.D., and Leopold Ärfors, M.D.
Göteborg and Stockholm, Sweden

Fetal blood group determination in first-trimester pregnancy for the management of severe immunization 120
Humphrey H. Kanhai, M.D., Jack Bennebroek Gravenhorst, M.D., Reinoud J. Gemke, M.D., Marijke A. Overbeeke, M.S., Luigi F. Bernini, M.D., and Geoffrey C. Beverstock, M.D.
Leiden and Amsterdam, The Netherlands

Chorionic biopsy in management of severe Kell alloimmunization 124
F. Rodesch, M.D., M. Lambermont, M.Sc., C. Donner, M.D., Ph. Simon, M.D., F. Romasco, B.S., J. Schwers, M.D., and J. Wybran, M.D.
Brussels, Belgium

Contents

Spontaneous rupture of the membranes, functional dystocia, oxytocin treatment, and the route of delivery 125
Joseph Seitchik, M.D., Alan E. C. Holden, M.A., and Maria Castillo, R.N.
San Antonio, Texas

Polyhydramnios and elevated intrauterine pressure during amnioinfusion 130
Bannie L. Tabor, M.D., and Jeffrey A. Maier, M.D.
Torrance, California

Screening for gestational diabetes: One-hour carbohydrate tolerance test performed by a virtually tasteless polymer of glucose 132
E. Albert Reece, M.D., Theodore Holford, Ph.D., Susan Tuck, M.D., Mitzi Bargar, R.N., Theresa O'Connor, M.P.H., and John C. Hobbins, M.D.
New Haven, Connecticut

Increased incidence of spontaneous abortion in pregnancies complicated by maternal diabetes mellitus 135
Hamish W. Sutherland, M.B., Ch.B., and Colin W. Pritchard, Ph.D.
Aberdeen, Scotland

Immunohistochemical studies on collagen types in the uterine cervix in pregnant and nonpregnant states 138
Toshinari Minamoto, M.D., Katsuhiko Arai, D.V.M., Shun Hirakawa, M.D., and Yutaka Nagai, Ph.D.
Tokyo, Japan

The planning of pregnancy among low-income women in central Harlem 145
Marie C. McCormick, M.D., Sc.D., J. Brooks-Gunn, Ph.D., Thomasine Shorter, M.S.W., Claudina Y. Wallace, M.D., M.P.H., John H. Holmes, B.S., and Margaret C. Heagarty, M.D.
Philadelphia, Pennsylvania, Princeton, New Jersey, and New York, New York

Ultrasonically evident fetal and nuchal skin thickening: Is it specific for Down syndrome? 150
Ants Toi, M.D., Gary F. Simpson, M.D., and Roy A. Filly, M.D.
Hamilton, Ontario, Canada, and San Francisco, California

Clinical high-risk designation does not predict excess fetal-maternal hemorrhage 154
Paul M. Ness, M.D., Michael L. Baldwin, M.T.(A.S.C.P.), S.B.B., and Jennifer R. Niebyl, M.D.
Baltimore, Maryland

Endometrial blood flow measured by xenon 133 clearance in women with normal menstrual cycles and dysfunctional uterine bleeding 158
Ian S. Fraser, M.D., Gay McCarron, R.N., Brian Hutton, M.Sc., and Daniel Macey, Ph.D.
Sydney and Camperdown, Australia, and Oxford, England

(Contents continued on page 14A)

Contents continued from page 13A

Routine hepatitis screening in adolescent pregnancies: Is it cost effective? 166
Ann M. Wetzel, M.D., and Donna S. Kirz, M.D.
Chicago, Illinois

Cardiac output response to prostaglandin E_2–induced abortion in the second trimester 170
Donald C. Willis, M.D., Donald Caton, M.D., J. Patrick Levelle, M.D., and Tina Banner, R.N.
Gainesville, Florida

Prostaglandin E_2 release on the fetal and maternal sides of the amnion and chorion-decidua before and after term labor 173
John A. McCoshen, Ph.D., Kimberly A. Johnson, B.Sc., Norman H. Dubin, Ph.D., and Ramesh B. Ghodgaonkar, B.Pharm.
Winnipeg, Manitoba, Canada, and Baltimore, Maryland

A prospective study to compare serum human placental lactogen and menstrual dates for determining gestational age 178
Paul G. Whittaker, D.Phil., Tom Lind, M.B., B.S., and Jacqueline Y. Lawson, B.Sc.
Newcastle upon Tyne, England

Similarities of Negro and Caucasian fetal extremity lengths in the interval from 9 to 20 weeks of pregnancy 183
Robert A. Munsick, M.D., Ph.D.
Indianapolis, Indiana

Academia—An endangered species 185
Douglas J. Marchant, M.D.
Boston, Massachusetts

Effect of low-dose aspirin on angiotensin II pressor response in human pregnancy 193
Luis Sanchez-Ramos, M.D., Mary Jo O'Sullivan, M.D., and Jose Garrido-Calderon, M.D.
Miami, Florida, and Santo Domingo, Dominican Republic

Deoxyribonucleic acid hybridization analysis for the detection of urogenital *Chlamydia trachomatis* infections in women 195
Chia C. Pao, Ph.D., Shyh-Shyan Lin, M.S., Tsyr-En Yang, M.S., Yung-Kuei Soong, M.D., Pai-Shun Lee, R.N., and Jung-Yaw Lin, Ph.D.
Taipei, Taiwan, Republic of China

Divergent effects of two low-dose oral contraceptives on sex hormone–binding globulin and free testosterone 199
Claudia Jung-Hoffmann, M.D., and Herbert Kuhl, Ph.D.
Frankfurt am Main, Federal Republic of Germany

Contents

Source of prenatal care and infant birth weight: The case of a North Carolina county 204
Paul A. Buescher, Ph.D., Clinton Smith, M.D., M.P.H., Joseph L. Holliday, M.D., M.P.H., and Ronald H. Levine, M.D., M.P.H.
Raleigh, North Carolina, Nashville, Tennessee, and Greensboro, North Carolina

Hairy cell leukemia in pregnancy: A case report 210
J. Kell Williams, M.D.
Detroit, Michigan

CURRENT INVESTIGATION

Sexually transmitted papillomaviral infections. I. The anatomic distribution and pathologic grade of neoplastic lesions associated with different viral types 212
Richard Reid, M.D., Mitchell Greenberg, M.D., A. Bennett Jenson, M.D., Mutajaba Husain, M.D., Jerry Willett, M.D., Yahya Daoud, M.S., Gary Temple, M.D., C. Robert Stanhope, M.D., Alfred I. Sherman, M.D., Garth D. Phibbs, M.D., and Attila T. Lorincz, Ph.D.
Detroit, Michigan, Washington, D. C., Gaithersburg, Maryland, Rochester, Minnesota, and Toledo, Ohio

BASIC SCIENCE SECTION

Yolk sac tumors of the ovary and the human yolk sac 223
Toshimitsu Takashina, M.D., Yuugi Kanda, M.D., Osamu Hayakawa, M.D., Ryuichi Kudo, M.D., Eiki Ito, M.D., and Satoru Sagae, M.D.
Sapporo, Hokkaido, Japan

Effects of maternal anemia on uteroplacental and fetal oxidative metabolism in sheep 230
Mary E. Paulone, M.D., Daniel I. Edelstone, M.D., and Ann Shedd, M.D.
Pittsburgh, Pennsylvania

Triiodothyronine receptors in porcine granulosa cells 237
Najib G. Wakim, M.D., Natarajan Ramani, Ph.D., and Ch. V. Rao, Ph.D.
Louisville, Kentucky

Epidermal growth factor deficiency during pregnancy causes abortion in mice 241
Osamu Tsutsumi, M.D., and Takami Oka, Ph.D.
Bethesda, Maryland

(Contents continued on page 16A)

Contents continued from page 15A

Intrauterine growth retardation after long-term maternal salicylate administration in the rabbit 245
Jeffrey C. Lukas, M.D., Ted S. Rosenkrantz, M.D., John R. Raye, M.D., Patricia J. Porte, and Anthony F. Philipps, M.D.
Farmington, Connecticut

A monoclonal antibody to a cell surface determinant in human endometrial epithelium: Stage-specific expression in the menstrual cycle 250
John D. Aplin, Ph.D., and Mourad W. Seif, M.D.
Manchester, England

Comparison of serum progesterone levels in dogs after administration of progesterone by vaginal tablet and vaginal suppositories 253
L. David Fulper, M.S., Robert W. Cleary, Ph.D., Ernest C. Harland, D.V.M., Ahmed H. Hikal, Ph.D., and Alan B. Jones, Ph.D.
University, Mississippi

CORRESPONDENCE

Papanicolaou smears in diagnosis of endocervical chlamydial infection G. E. Forster, I. Cookey, M.B., P. I. Richman, M.B., R. Jha, M.D., D. Coleman, M.D., P. E. Munday, M.D., B. J. Thomas, Ph.D., D. A. Hawkins, R. T. Evans, M.I.Biol., and D. Taylor-Robinson, M.D. *London and Harrow, Middlesex, England* 257

Midforceps deliveries Alex S. Tulsky, M.D. *Chicago, Illinois* 257

Reply LeRoy J. Dierker, Jr., M.D. *Cleveland, Ohio* 257

Preeclampsia M. P. Moore, M.B., and C. W. G. Redman, M.B. *Christchurch, New Zealand, and Headington, Oxford, England* 257

Reply Baha M. Sibai, M.D. *Memphis, Tennessee* 258

Fetal lung maturity William R. Walker, M.D., and Nicolas V. Simon, M.D. *York, Pennsylvania* 259

Reply David P. Kogon, M.D. *Amherst, Nova Scotia, Canada* 259

Preterm labor Jefrey Arlen, M.D. *Denver, Colorado* 260

Reply Robert K. Creasy, M.D., and Bernard Gonik, M.D. *Houston, Texas* 260

Ritodrine or terbutaline—The optimal dosage in the treatment of preterm labor Göran Berg, M.D., and Gunnar Rydén, M.D. *Linköping, Sweden* 260

Effect of calcium supplementation on the vascular sensitivity to angiotensin II in pregnant women Patricio Lopez-Jaramillo, M.S., M.D., Marcelo Narvaez, M.D., and Rodrigo Yepez, M.D. *Quito, Ecuador* 261

Cesarean sections Henry J. Soldati, M.D. *San Francisco, California* 262

Contents

ANNOUNCEMENT

Changes in definitions of clinical staging for carcinoma of the cervix and ovary: International Federation of Gynecology and Obstetrics 263

ROSTER

Roster of American obstetric and gynecologic societies 265

BOOKS

Books received 267

ITEMS

Items 268

CORRECTIONS

Predicting acute pelvic inflammatory disease: A multivariate analysis (Hadgu A, Westrom L, Brooks CA, Reynolds GH, Thompson SE. 1986;155:954-60) 264

Intestinal injury in gynecologic surgery: A ten-year experience (Krebs H-B. 1986;155:509-14) 264

Information for authors on pages 27A, 28A, and 29A

Index to advertisers on page 62A

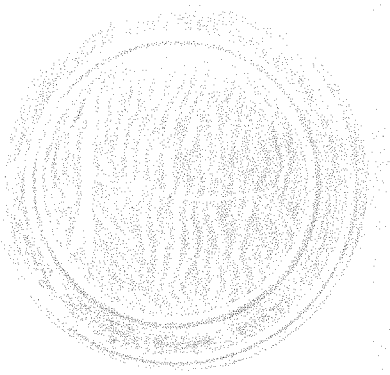
Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1987 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 156 (January-June) and Vol. 157 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by The C. V. Mosby Company for libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that 10¢ per page is paid directly to CCC, 29 Congress St., Salem, MA 01970; phone (617)744-3350. The U.S. Government retains a nonexclusive, royalty-free license in and to any copyright on articles authored by government employees. 0002-9378/87 $00.00 + .10.

If your patient is estrogen deficient, she needs proven results a transdermal can't deliver...



She needs



PREMARIN®

(Conjugated Estrogens Tablets)

Only oral estrogen provides

- Proven protection against osteoporosis
- Decreased total cholesterol; increased HDL cholesterol; lowered LDL cholesterol
- Easy cycling with progestin
- Unsurpassed long-term safety

PREMARIN®

Complete estrogen therapy

Please see following page for brief summary of prescribing information.

The appearance of PREMARIN Tablets is a trademark of Ayerst Laboratories.

BRIEF SUMMARY *(FOR FULL PRESCRIBING INFORMATION AND PATIENT INFORMATION, SEE PACKAGE CIRCULAR.)*

PREMARIN® Brand of conjugated estrogens tablets, USP

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent, case-controlled studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than one year. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade. The three case-controlled studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semi-annual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.

The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing, in later life, a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1,000 exposures. Furthermore, a high percentage of such exposed women (from 30% to 90%) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb-reduction defects. One case-controlled study estimated a 4.7-fold increased risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than 1 per 1,000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. If PREMARIN is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the advisability of pregnancy continuation.

DESCRIPTION: PREMARIN (conjugated estrogens tablets, USP) for oral administration contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters. Tablets are available in 0.3 mg, 0.9 mg, 1.25 mg, and 2.5 mg strengths of conjugated estrogens.

INDICATIONS AND USAGE: Moderate to severe *vasomotor* symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression without associated vasomotor symptoms and they should not be used to treat such conditions.) Osteoporosis (abnormally low bone mass). Atrophic vaginitis. Kraurosis vulvae. Female castration.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

Concomitant Progestin Use: The lowest effective dose appropriate for the specific indication should be utilized. Studies of the addition of a progestin for 7 or more days of a cycle of estrogen administration have reported a lowered incidence of endometrial hyperplasia. Morphological and biochemical studies of the endometrium suggest that 10 to 13 days of progestin are needed to provide maximal maturation of the endometrium and to eliminate any hyperplastic changes. Whether this will provide protection from endometrial carcinoma has not been clearly established. There are possible additional risks which may be associated with the inclusion of progestin in estrogen replacement regimens. (See PRECAUTIONS.) The choice of progestin and dosage may be important; product labeling should be reviewed to minimize possible adverse effects.

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 2. Known or suspected estrogen-dependent neoplasia. 3. Known or suspected pregnancy (see Boxed Warning). 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

WARNINGS: Estrogens have been reported to increase the risk of endometrial carcinoma (see Boxed Warning). However, a recent large, case-controlled study indicated no increase in risk of breast cancer in postmenopausal women. A recent study has reported a 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease in women receiving postmenopausal estrogens.

Adverse effects of oral contraceptives may be expected at the larger doses of estrogen used to treat prostatic or breast cancer or postpartum breast engorgement; it has been shown th there is an increased risk of thrombosis in men receiving estrogens for prostatic cancer a women for postpartum breast engorgement. Users of oral contraceptives have an increased ri of diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarctio Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported oral contraceptive users. An increased risk of postsurgery thromboembolic complications h also been reported in users of oral contraceptives. If feasible, estrogen should be discontinue at least 4 weeks before surgery of the type associated with an increased risk of thromboer bolism, or during periods of prolonged immobilization. Estrogens should not be used in perso with active thrombophlebitis, thromboembolic disorders, or in persons with a history of su disorders in association with estrogen use. They should be used with caution in patients wi cerebral vascular or coronary artery disease. Large doses (5 mg conjugated estrogens per da comparable to those used to treat cancer of the prostate and breast have been shown increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebiti When doses of this size are used, any of the thromboembolic and thrombotic adverse effec should be considered a clear risk.

Benign hepatic adenomas should be considered in estrogen users having abdominal pa and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has bee reported in women taking estrogen-containing oral contraceptives. Increased blood pressu may occur with use of estrogens in the menopause and blood pressure should be monitore with estrogen use. A worsening of glucose tolerance has been observed in patients c estrogen-containing oral contraceptives. For this reason, diabetic patients should be careful observed. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bor metastases.

PRECAUTIONS: Physical examination and a complete medical and family history should b taken prior to the initiation of any estrogen therapy with special reference to blood pressur breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a gener rule, estrogen should not be prescribed for longer than one year without another physic examination being performed. Conditions influenced by fluid retention, such as asthma, ep lepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patien may develop manifestations of excessive estrogenic stimulation, such as abnormal or exce sive uterine bleeding, mastodynia, etc. Prolonged administration of unopposed estroge therapy has been reported to increase the risk of endometrial hyperplasia in some patients. Or contraceptives appear to be associated with an increased incidence of mental depressio Patients with a history of depression should be carefully observed. Pre-existing uterin leiomyomata may increase in size during estrogen use. The pathologist should be advised estrogen therapy when relevant specimens are submitted. If jaundice develops in any patie receiving estrogen, the medication should be discontinued while the cause is investigate Estrogens should be used with care in patients with impaired liver function, renal insufficienc metabolic bone diseases associated with hypercalcemia, or in young patients in whom bon growth is not yet complete. If concomitant progestin therapy is used, potential risks may includ adverse effects on carbohydrate and lipid metabolism.

The following changes may be expected with larger doses of estrogen:

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increase norepinephrine-induced platelet aggregability.

c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroi hormone, as measured by PBI, T_4 by column, or T_4 by radioimmunoassay. Free T_3 resin uptak is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered.

d. Impaired glucose tolerance.

e. Decreased pregnanediol excretion.

f. Reduced response to metyrapone test.

g. Reduced serum folate concentration.

h. Increased serum triglyceride and phospholipid concentration.

As a general principle, the administration of any drug to nursing mothers should be done onl when clearly necessary since man drugs are excreted in human milk.

Long-term, continuous administra tion of natural and synthetic estro gens in certain animal species in creases the frequency of carcinoma of the breast, cervix, vagina, and live However, in a recent, large case-con trolled study of postmenopausa women there was no increase in risk of breast cancer with use of conju gated estrogens.

ADVERSE REACTIONS: The fol lowing have been reported with es trogenic therapy, including oral con traceptives: breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea; pre menstrual-like syndrome; amenorrhea during and after treatment; increase in size of uterin fibromyomata; vaginal candidiasis, change in cervical erosion and in degree of cervical secre tion; cystitis-like syndrome; tenderness, enlargement, secretion (of breasts); nausea, vomiting abdominal cramps, bloating; cholestatic jaundice; chloasma or melasma which may persis when drug is discontinued; erythema multiforme; erythema nodosum; hemorrhagic eruption loss of scalp hair; hirsutism; steepening of corneal curvature; intolerance to contact lenses headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea, and withdrawal bleeding may occur in females

DOSAGE AND ADMINISTRATION: 1. *Given cyclically for short-term use only.* For treatmen of moderate-to-severe *vasomotor* symptoms, atrophic vaginitis, or kraurosis vulvae associate with the menopause (0.3 mg to 1.25 mg or more daily).

The lowest dose that will control symptoms should be chosen and medication should b discontinued as promptly as possible.

Administration should be cyclic (eg, three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three- to six-month intervals.

2. *Given cyclically:* Osteoporosis. Female castration.

Osteoporosis —0.625 mg daily. Administration should be cyclic (eg, three weeks on and one week off).

Female castration—1.25 mg daily cyclically. Adjust upward or downward according to re sponse of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Patients with an intact uterus should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

PREMARIN®

(Conjugated Estrogens Tablets)

Complete estrogen therapy

Ayerst® AYERST LABORATORIES
New York, NY 10017

6339/1286

American Journal of Obstetrics and Gynecology

Editors

JOHN I. BREWER, *Editor in Chief*
Sandra Fernandez, *Senior Editorial Assistant*

FREDERICK P. ZUSPAN, *Editor*
Meta M. Peterson, *Editorial Assistant*

E. J. QUILLIGAN, *Editor*
Rosalyn Vu, *Editorial Assistant*

ALBERT B. GERBIE, *Associate Editor*

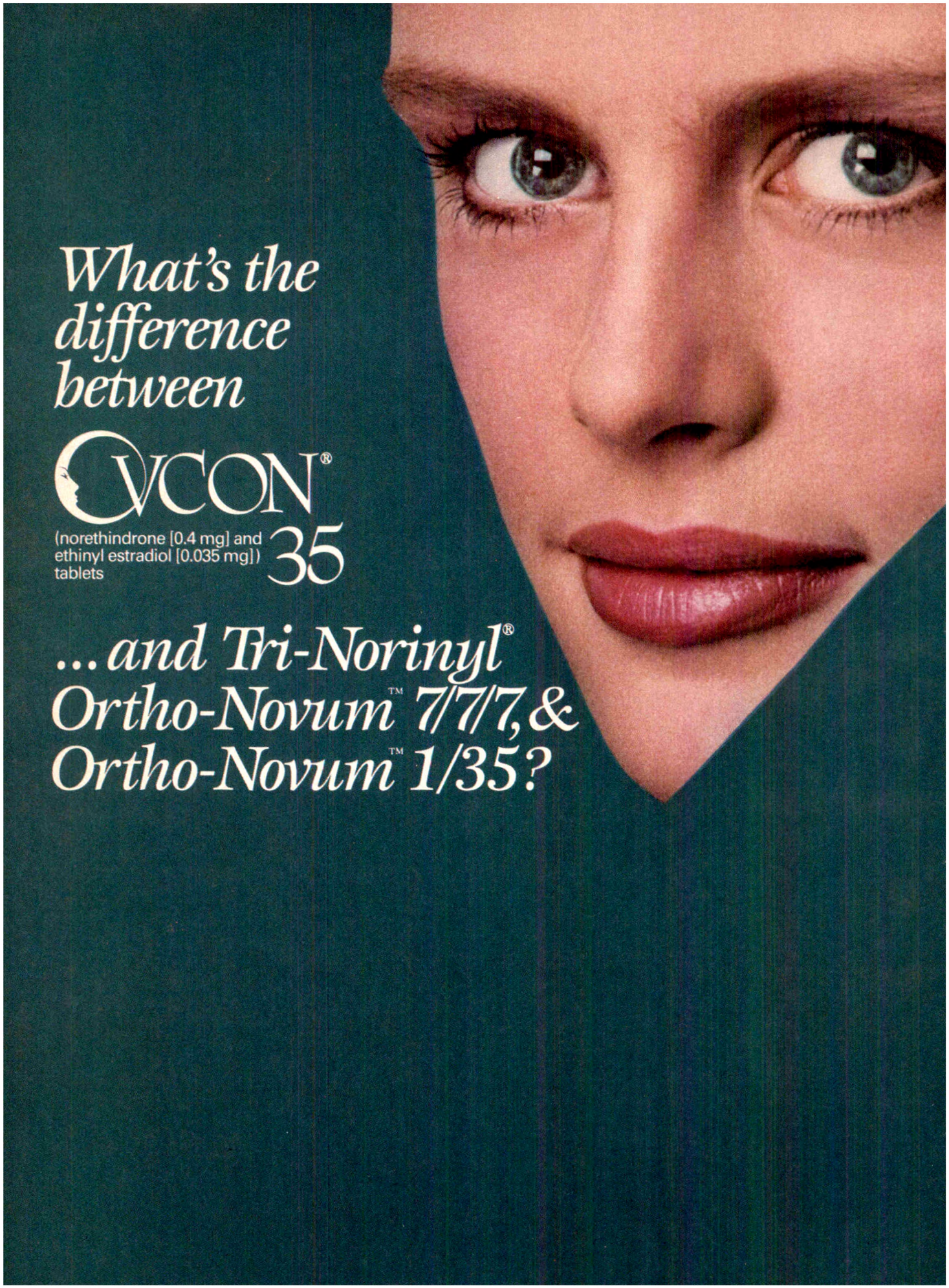
Advisory committee on policy

William R. Anderson
Mason C. Andrews
E. Forrest Boyd
Amelia Cruz
Leo J. Dunn
A. Jerome Freeland
Morrie M. Gelfand
T. Terry Hayashi
Edgar O. Horger III
Hobart C. Hortman
Walter S. Keifer
Charles D. Kimball
Roger A. Lobo
Douglas J. Marchant
James H. Maxwell
Arthur M. McCausland
James A. Merrill
Frank C. Miller
Patrick T. Mohide
John C. Morrison
Richard Paul
Keith P. Russell
Ruth W. Schwartz
Joseph C. Scott, Jr.
Ruth Stockdale
Henry A. Thiede
Edward E. Wallach
Anne C. Wentz

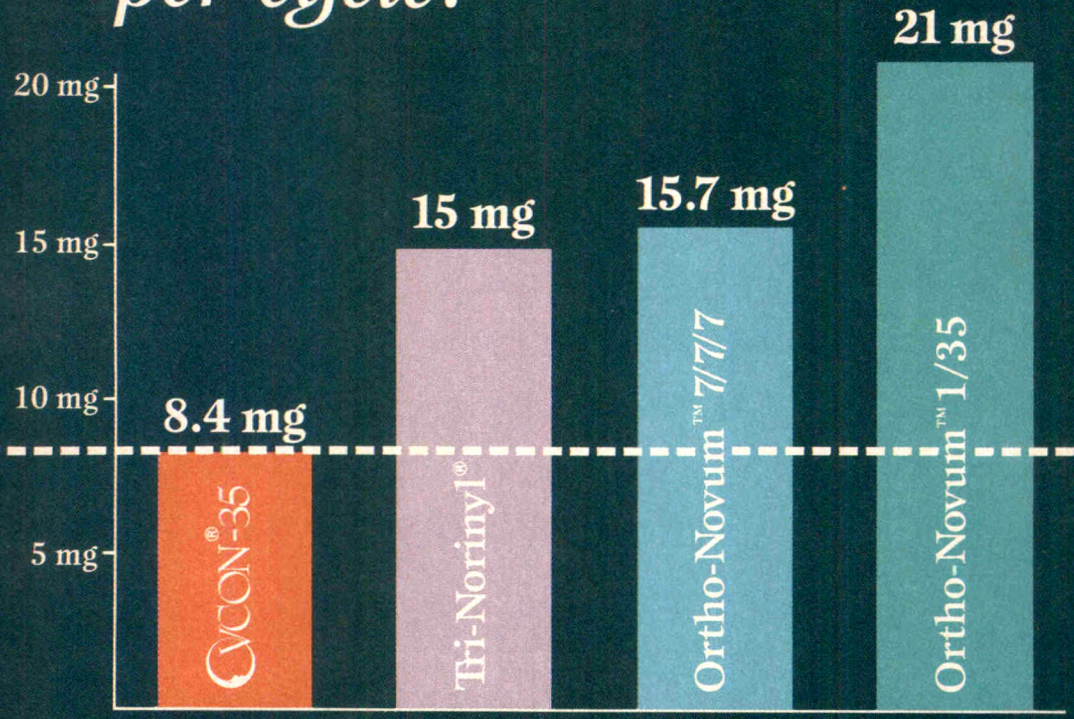
Board of corresponding editors

Stuart Campbell, *England*
Luigi Carenza, *Italy*
Eliahu Sadovsky, *Israel*
Nils-Otto Sjöberg, *Sweden*
Masakuni Suzuki, *Japan*

Publisher
THE C. V. MOSBY COMPANY, 11830 Westline Industrial Drive, St. Louis, Missouri 63146
Kay G. Goehler, *Senior Manuscript Editor*


What's the difference between
OVCON®
(norethindrone [0.4 mg] and ethinyl estradiol [0.035 mg]) tablets
35
...and Tri-Norinyl®
Ortho-Novum™ 7/7/7,&
Ortho-Novum™ 1/35?

they contain 78% to 150% more norethindrone per cycle!



Amounts of norethindrone per cycle

the OC with the lowest norethindrone dose

Ovcon®-35

(norethindrone [0.4 mg] and ethinyl estradiol [0.035 mg]) tablets

Mead Johnson

OVCON®-50

(norethindrone [1 mg] and ethinyl estradiol [0.05 mg]) tablets

OVCON®-35

(norethindrone [0.4 mg] and ethinyl estradiol [0.035 mg]) tablets

Indications and Usage: Ovcon is indicated for the prevention of pregnancy. Some combination products containing 35 mcg. or less of ethinyl estradiol report slightly higher pregnancy rates than are reported for the higher dose combination products.

Dose-related Risk of Thromboembolism from Oral Contraceptives: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing 50 mcg. or less of estrogen.

Contraindications: Oral contraceptives should not be used in women with any of the following conditions: 1) Thrombophlebitis or thromboembolic disorders. 2) A past history of deep vein thrombophlebitis or thromboembolic disorders. 3) Cerebral vascular or coronary artery disease. 4) Known or suspected carcinoma of the breast. 5) Known or suspected estrogen dependent neoplasia. 6) Undiagnosed abnormal genital bleeding. 7) Known or suspected pregnancy. 8) Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

Warnings:

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. Women who use oral contraceptives should be strongly advised not to smoke. Use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1) *Thromboembolic Disorders and Other Vascular Problems.* An increased risk of thromboembolic and thrombotic disease associated with use of oral contraceptives is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. In a study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater. An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported. Studies conducted in the United Kingdom found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction compared to nonusers who do not smoke. Oral contraceptive users who smoke have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. The amount of smoking is a very important factor. British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives; preparations containing 100 mcg. or more of estrogen were associated with a higher risk of thromboembolism than those containing 50–80 mcg. of estrogen. The relative risk of thromboembolic disease associated with progestin-only oral contraceptives has not been determined. Cases of thromboembolic disease have been reported in women using progestin-only products, and they should not be presumed to be risk-free. The overall excess mortality rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15–34, 5/100,000; ages 35–44, 33/100,000; ages 45–49, 140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. The highest risk was found in heavy cigarette smokers (15 or more cigarettes per day) who used oral contraceptives and were aged 40 or older. Women who smoke should be advised not to use oral contraceptives. The use of oral contraceptives in women over age 40 with other risk factors is not recommended. The mortality associated with all of the methods of birth control is low compared to the risk of childbirth, with the exception of oral contraceptive users who smoke and are over age 40. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and for myocardial infarction is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia, and especially by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. If feasible, oral contraceptives should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2) *Ocular Lesions.* There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue the medication if there is unexplained sudden or gradual, partial or complete loss of vision; sudden onset of proptosis or diplopia; papilledema; or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3) *Carcinoma.* Long term administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. In humans, one study investigated the first 21 cases of endometrial adenocarcinoma in women on oral contraceptives reported to a registry. Of those women without predisposing risk factors for this disease, nearly all occurred in women who had used a sequential oral contraceptive. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestin-only oral contraceptives. No increase in breast cancer in women taking oral contraceptives has been reported although one study reported an increased risk of breast cancer in subgroups of women treated using oral contraceptives with documented benign breast disease. There is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives. 4) *Hepatic Adenoma.* Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study reported a higher risk associated with oral contraceptive formulations with high hormonal potency. Although benign and rare, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short term as well as long term users of oral contraceptives. Two studies relate the risk with duration of contraceptive use, the risk being much greater after 4 or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5) *Use in Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring.* Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. A high percentage of such exposed women (30% to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Similar data are not available with the use of other estrogens but it cannot be presumed that they would not induce similar changes. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications and there is no evidence from well-controlled studies that progestins are effective for these uses. Increases in chromosomal aberrations have been reported in women who become pregnant soon after ceasing oral contraceptive therapy. Embryos with these anomalies are virtually always spontaneously aborted. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed the patient should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation should be discussed in light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months. Administration of progestin-only or progestin-estrogen combinations to induce withdrawal bleeding should not be used as a test for pregnancy. 6) *Gall Bladder Disease.* Studies have reported an increased risk of surgically confirmed gallbladder disease appearing after one year of oral contraceptive use and doubling of the risk after 4 or 5 years of use. 7) *Carbohydrate and Lipid Metabolic Effects.* A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. Increased serum levels of triglycerides and total phospholipids have been observed in oral contraceptive users. The clinical significance of this observation is unknown at this time. 8) *Elevated Blood Pressure.* An increase in blood pressure has been reported in women receiving oral contraceptives. The prevalence of hypertension in oral contraceptive users may be no higher than nonusers in the first year of oral contraceptive use but increases with longer exposure and in the fifth year of use is two and one-half to three times the reported prevalence in the first year. Women who previously had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. 9) *Headaches.* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of oral contraceptives and investigation of the cause. 10) *Bleeding Irregularities.* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. 11) *Ectopic Pregnancy.* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in oral contraceptive failures, the ratio of ectopic to intrauterine pregnancies is higher than in women not using oral contraceptives since the drugs are more effective in preventing intrauterine rather than ectopic pregnancy. The higher ectopic-intrauterine ratio has been reported with both combination products and progestin-only oral contraceptives. 12) *Breast Feeding.* A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

Precautions: 1) A complete medical and family history should be taken prior to the initiation of oral contraceptives. Examination should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than 1 year without another physical examination being performed. 2) Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3) Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 4) Because oral contraceptives may cause some degree of fluid retention, conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, cardiac or renal insufficiency or asthma require careful observation. 5) Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6) Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7) Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8) Serum folate levels may be depressed by oral contraceptive therapy. This may complicate subsequent pregnancy with regard to folate deficiency. 9) The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10) Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T_4 by column, or T_4 by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG, free T_4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. 11) The active yellow tablets and the inert green tablets in Ovcon-50 (21 and 28 day regimens) and the inert green tablets in the 28 day regimen of Ovcon-35 contain FD&C Yellow No. 5 (tartrazine) which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals. Although the overall incidence of FD&C Yellow No. 5 (tartrazine) sensitivity in the general population is low, it is frequently seen in patients who also have aspirin hypersensitivity.

Information for the Patient: Detailed Patient Labeling has been prepared for use by the patient and has been made available for distribution by the pharmacist.

Drug Interactions: Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Adverse Reactions: An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **Warnings**): Thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis; benign hepatomas; neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle (other reactions, as a general rule, are seen much less frequently or only occasionally); gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes (tenderness, enlargement, and secretion); change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; changes in corneal curvature (steepening), intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

Acute Overdosage: Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

03

Mead Johnson LABORATORIES

C-V21
MJL 6-4184

Bristol-Myers U.S. Pharmaceutical and Nutritional Group • Evansville, Indiana 47721 USA

American Journal of Obstetrics and Gynecology

Societies

in addition to those listed on the front cover
the Journal is the official publication
of the following societies

NEW YORK OBSTETRICAL SOCIETY

OBSTETRICAL SOCIETY OF PHILADELPHIA

BROOKLYN GYNECOLOGICAL SOCIETY

ST. LOUIS GYNECOLOGICAL SOCIETY

NEW ORLEANS GYNECOLOGICAL AND OBSTETRICAL SOCIETY

THE OBSTETRICAL AND GYNECOLOGICAL SOCIETY OF MARYLAND

CHICAGO GYNECOLOGICAL SOCIETY

CINCINNATI OBSTETRICAL AND GYNECOLOGICAL SOCIETY

WASHINGTON GYNECOLOGICAL SOCIETY

PITTSBURGH OBSTETRICAL AND GYNECOLOGICAL SOCIETY

BOSTON OBSTETRICAL SOCIETY

LOUISVILLE OBSTETRICAL AND GYNECOLOGICAL SOCIETY

SEATTLE GYNECOLOGICAL SOCIETY

ALABAMA ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

AKRON OBSTETRICAL AND GYNECOLOGICAL SOCIETY

KANSAS CITY GYNECOLOGICAL SOCIETY

CENTRAL NEW YORK ASSOCIATION OF GYNECOLOGISTS
AND OBSTETRICIANS

NEW JERSEY OBSTETRICAL AND GYNECOLOGICAL SOCIETY

IOWA OBSTETRIC AND GYNECOLOGIC SOCIETY

THE TEXAS ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

OKLAHOMA CITY OBSTETRICAL AND GYNECOLOGICAL SOCIETY

MEMPHIS OBSTETRICAL AND GYNECOLOGICAL SOCIETY

UTAH OBSTETRICAL AND GYNECOLOGICAL SOCIETY

ROCHESTER OBSTETRICAL AND GYNECOLOGICAL SOCIETY

ARKANSAS OBSTETRICAL AND GYNECOLOGICAL SOCIETY

TENNESSEE STATE OBSTETRICAL AND GYNECOLOGICAL SOCIETY

NEW YORK GYNECOLOGICAL SOCIETY

PACIFIC NORTHWEST OBSTETRICAL AND GYNECOLOGICAL ASSOCIATION

BUFFALO OBSTETRICAL AND GYNECOLOGICAL SOCIETY

SAN FRANCISCO GYNECOLOGICAL SOCIETY

JACKSON GYNECIC SOCIETY

INDIANA OBSTETRICAL AND GYNECOLOGICAL SOCIETY

THE MINNESOTA OBSTETRICAL AND GYNECOLOGICAL SOCIETY

High Risk Obstetrics, April 2–4, 1987
Specialty Review in Obstetrics and Gynecology, April 20–25, 1987

Our practice is limited to CME



By limiting your practice to Ob/Gyne, you provide your patients with the expertise that comes with specialization. Likewise, our specialization has made us experts in CME. Discover why your colleagues have placed their confidence in us. Discover The Graduate School — Your CME Specialist.

To register for High Risk Obstetrics or Specialty Review in Ob/Gyne or for additional information call toll-free

1-800-621-4651

in Illinois 1-800-621-4649

THE COOK COUNTY GRADUATE SCHOOL OF MEDICINE
707 South Wood St.
Chicago, IL 60612

THE FIRST INTERNATIONAL CONFERENCE ON

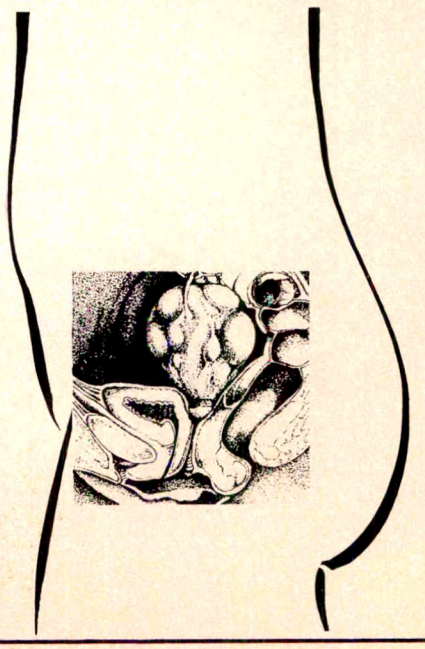
VAGINAL SURGERY:

Today's and Tomorrow's Techniques and Choices

Friday and Saturday,
March 6-7, 1987
St. Louis, Missouri
Doubletree Hotel and Conference Center

Physicians of renown will report on their personal experiences with various vaginal surgical techniques and their reasons for selecting and performing vaginal surgery.

This unique—not to be missed—conference will focus on far-reaching practical implications of vaginal surgery for the clinician to augment his/her surgical skills in a changing and demanding marketplace.



Presented by St. John's Mercy Medical Center, St. Louis, Missouri

For additional information contact: Judith L. O'Guin
• Corporate Meeting Planner • St. John's Mercy Medical Center
• 615 South New Ballas Road • St. Louis, Missouri 63141
• Telephone: 314-569-6290

Information for Authors

American Journal of Obstetrics and Gynecology

Copyright © 1987 by The C. V. Mosby Company

Editors

John I. Brewer, *Editor in Chief*
710 North Fairbanks Court, Chicago, IL 60611

Sandra Fernandez, *Senior Editorial Assistant*
Tel.: (312) 908-5266

Frederick P. Zuspan, *Editor*
The Ohio State University, Fifth Floor, Means Hall, 1654 Upham Dr., Columbus, OH 43210

Meta M. Peterson, *Editorial Assistant*
Tel.: (614) 293-8798

E. J. Quilligan, *Editor*
University of California, Davis, School of Medicine, Department of Obstetrics and Gynecology, 1621 Alhambra Blvd., Suite 2500, Sacramento, CA 95816

Rosalyn Vu, *Editorial Assistant*
Tel.: (916) 453-5950

Albert B. Gerbie, *Associate Editor*
710 North Fairbanks Court, Chicago, IL 60611

Publisher
THE C. V. MOSBY COMPANY, 11830 Westline Industrial Drive, St. Louis, Missouri 63146

Kay G. Goehler, *Senior Manuscript Editor*
Tel.: (314) 872-8370

INFORMATION FOR AUTHORS

Editorial policies

The requirements for manuscripts submitted to the *American Journal of Obstetrics and Gynecology* conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors and published in *Annals of Internal Medicine* 1982;96(6):766-71. Certain requirements unique to our JOURNAL are provided in Information for Authors, published in each issue of the JOURNAL, and in more detail in the *Guide to Writing for the American Journal of Obstetrics and Gynecology*. The latter may be obtained from The C. V. Mosby Company or the Editors on request.

Manuscripts should be submitted to one of the three Editors as follows: (1) Dr. Brewer—all articles originating from the southeastern quadrant of the United States or Canada and those presented before one of the official sponsoring societies; (2) Dr. Zuspan—articles from the northeastern quadrant of the United States, Japan, Israel, Italy, and England, articles written for Clinical Opinion and Current Development, and Letters to the Editors; (3) Dr. Quilligan—articles from the north central states (including Ohio), states west of the Mississippi, Hawaii, Alaska, and abroad (except Japan, Israel, Italy, and England).

Most of the provisions of the Copyright Act of 1976 became effective on January 1, 1978. **Therefore, all manuscripts must be accompanied by the following written statement, signed by one author:** "The undersigned author transfers all copyright ownership of the manuscript [title of article] to The C. V. Mosby Company in the event the work is published. The undersigned author warrants that the article is original, is not under consideration by another journal, and has not been previously published. I sign for and accept responsibility for releasing this material on behalf of any and all co-authors." Authors will be consulted, when possible, regarding republication of their material.

When submitting a manuscript, an author should include, if applicable, a full statement about all submissions and prior reports in any medium that might be regarded as prior or duplicate publication of the same or similar work. Copies of such material should be included with the submitted manuscript. Articles previously published in another language will not be considered.

It is assumed by the Editors that articles emanating from a particular institution are submitted with the approval of the requisite authority. Articles dealing with human experimentation that require local institutional approval must have this approval prior to submission of the article for publication and it should be so indicated in the Methods section. Reports of experiments on animals must indicate which guidelines for the care and use of the animals were followed.

Statements and opinions expressed in articles and communications herein are those of the author(s) and not necessarily those of the Editor(s) or Publisher and the Editor(s) and Publisher disclaim any responsibility or liability for such material. Neither the Editor(s) nor the Publisher guarantees, warrants, or endorses any product or service advertised in this publication, nor do they guarantee any claim made by the manufacturer of such product or service.

General requirements for preparation of manuscripts

The original and two good-quality copies of the manu-

script and three glossy prints of illustrations are required.

Manuscripts must be typed double spaced on one side only on 22 by 28 cm (8 1/2 by 11 inch) white bond paper with 1 inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, abstract, body of text, references, legends, and tables.

Title page. The title page should contain in sequence the title (concise and suitable for indexing purposes); author line with first name, middle initial, and last name of all authors and their highest academic degree (both M.D. and Ph.D. are acceptable); city(ies), state(s) in which the study was conducted; divisional, or departmental, and institutional affiliations at the time the study was performed; acknowledgement of source(s) of support; presented line, if applicable; disclaimers, if any; name and address of author to whom requests for reprints should be addressed (if reprints will not be available, it should be so stated); and name and address of author responsible for correspondence concerning the manuscript if different from author to whom reprint requests are addressed. At the bottom of the title page supply a short title for the running head not exceeding 52 characters (including word spaces).

Abstract page and key words/phrases. On manuscript page two is the abstract, also typed double spaced with the required margins and headed by the title of the article and author(s) name(s). Abstracts for regular articles, Current Investigation, Clinical Opinion, and Current Development may not exceed 150 words. Abstracts for case reports and brief communications may not exceed 50 words. Below the abstract list 3 to 5 *key words* or short phrases for indexing purposes.

Text. Do not hesitate to write your manuscript in the first-person, active voice if it is more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations since the emphasis is on the "action" rather than the person performing the action.

Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors*. Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify Material and Methods, Results, and Comment. Authors may wish to summarize their findings in a short paragraph at the end of the Comment section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods section* describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results section* present the detailed findings. Include mentions of all tables and/or figures. Avoid duplication of text and supporting material. Emphasize only your important observations; do not compare your observations to those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment section* state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgements. Acknowledge only persons who have made substantive contributions to the study.

References. Limit of 16 references, except for case reports and brief communications, limited to 2, and Current Development, for which there is no limit. Number references consecutively in the order in which they are mentioned in the text. Use the format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals." Journal titles should conform to abbreviations used in *Cumulated Index Medicus*.

Illustrations and tables. A reasonable number of halftone and line illustrations will be reproduced without charge, but special arrangements must be made with the Editors for color reproduction. For color photographs, submit original transparencies and two sets of unmounted prints on glossy paper with good color balance. For black and white illustrations, submit three sets of 3 by 4 inch (minimum) to 5 by 7 inch (maximum) unmounted glossy photographic prints. Figures must be cited consecutively in the text in Arabic numerals and identified thusly on the back of the print (gummed label with author(s) name(s), title of article, number, and top marked clearly). Do not use paper clips. Legends must accompany all figures; type double spaced on a single sheet of paper if possible and number with manuscript in sequence after the references. Titles should be included in the legend, *not* on the print.

All lettering must be done with commercially available paste-on letters (or numbers) or by a professional; typed or freehand lettering is not acceptable. All lettering must be in proportion to the drawing, graph, or photograph. Original drawings, appropriately done in black India ink, roentgenograms, and other material must be submitted as glossy photographic prints with good black and white contrast. Consistency in size within the article is strongly preferred. Any special instructions regarding sizing should be clearly noted. Illustrations will be returned only on the author's request.

Tables should be typed on separate sheets of paper and included at the end of the text, one to a page. They should be numbered in Roman numerals. Each table must be cited

Estimating length of manuscript

The length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A 4200 word text can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7 page article in the JOURNAL. The 2000 words of text alone make approximately 8 pages of manuscript typed double spaced with the required 1 inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 typed words in manuscript. Thus if a greater number of illustrations and tables are used, the length of the text should be adjusted accordingly.

in sequence at an appropriate point in the text. Captions should be brief yet indicate clearly the purpose or content of each table and each column precisely defined by headings. Abbreviations and special designations should be explained in a footnote to the table. Special arrangements must be made with the Editors for elaborate tables because of space limitations.

Illustrations and tables should supplement, not duplicate, the text; presentation in either one or the other will suffice.

Permissions and photographic consent. Previously published tables and figures must be correctly referenced and written permission from the copyright holder included with the manuscript. Photographs of identifiable persons must be accompanied by signed releases or else all recognizable features masked.

Requirements for special sections

Case reports and brief clinical and basic science communications. Limit of 700 words, 2 references. Include abstract of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures are used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

Current Investigation. Same requirements as for a regular article.

Clinical Opinion. Limit of 3000 words, 16 references. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Current Development. Limit of 6000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Correspondence. Two types of correspondence will be considered for publication. (1) A Letter to the Editors commenting on an article that has appeared in the JOURNAL should be brief and directly related to the published article. The editorial staff reserves the right to shorten letters if necessary and to make minor editorial alterations without reference to the writer. Letters may be published together with a reply from the original author. As space for letters is limited, only a selection of letters submitted may be published. (2) A brief case presentation or a short report of a pertinent observation in the form of a Letter to the Editors will be considered for publication. All letters should be typed double spaced. Letters should be sent to Dr. Zuspan.

Announcements. Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

Books. Books received will be listed in the JOURNAL. They should be sent to Dr. Gerbie. No books will be returned.

Reprints. Reprints of articles must be obtained from the author. The corresponding author will receive a price schedule and order form at the time of publication. Reprints in quantities must be ordered from the Publisher with the author's consent.

Business communications. Communications of a business nature and all advertising communications should be addressed to: Journal Publisher, The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, or call Journal Advertising Production Manager (314) 872-8370.

Checklist

—Letter of submission
—Copyright transfer letter
—Original and two xerographic copies of manuscript
—Title page
—Title of article
—Full name(s) and affiliations of author(s)
—Author to whom correspondence is to be sent
—Reprint request line or line stating reprints not available
—Short title
—Abstract (double spaced), 3 to 5 key words/phrases
—Article proper (double spaced)
—References (double spaced), on a separate sheet
—Legends (double spaced), on a separate sheet
—Tables (double spaced), each on a separate sheet
—Illustrations, properly labeled (three copies of glossy prints)
—Permission to reproduce published material

TRI-LEVLEN

Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

A LOW-DOSE TRIPHASIC OC WITH THE CONFIDENCE THAT COMES FROM EXPERIENCE



*Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. The physician should remain alert to the earliest manifestations of any symptom of serious disease and discontinue oral contraceptive therapy when appropriate. These da represent only the results of selected studies. Therefore, before prescribing, the physician should read and remain alert to all data found in the complete prescribing information for the product. Please also see brief summary of prescribing information on the last page of advertisement.

A formulation with the lowest total hormonal dose in a combination OC

Phased doses of both levonorgestrel and ethinyl estradiol

Permits a decrease in total steroid dose without compromising effectiveness.

Low incidence of breakthrough bleeding

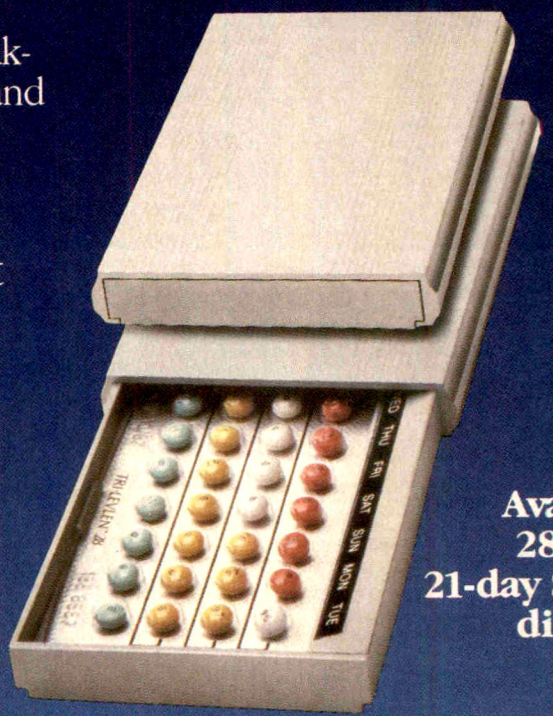
A low 5.7% overall incidence of breakthrough bleeding based on 77 U.S. and U.K. studies involving 3,546 women (35,036 total cycles).[1]

Simple first dose/first day start

The first dose is taken on the first day of menstruation. No need to count days or wait for Sunday starts.

Minimal impact on the following metabolic and hemodynamic parameters*

Lipids;[2,3] carbohydrate metabolism[2,3] and blood pressure[1] were not significantly affected in certain specific studies.



A formulation with the confidence of 170 million cycles of experience worldwide

Available in 28-day and 21-day Slidecase™ dispensers

TRI·LEVLEN™28 TRI·LEVLEN™21

BERLEX™ **Dedicated to innovation in OB/GYN medicine**

TRI·LEVLEN™ 28
TRI·LEVLEN™ 21

Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

BRIEF SUMMARY

Tri-Levlen™—6 brown tablets, each containing 0.050 mg of levonorgestrel (d(-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxygon-4-en-3-one), a totally synthetic progestogen, and 0.030 mg of ethinyl estradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3, 17-diol); 5 white tablets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol; 10 light-yellow tablets, each containing 0.125 mg levonorgestrel and 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day triphasic regimen).

Indications and Usage—Tri-Levlen Tablets are indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OCs) as a method of contraception.

Contraindications—OCs should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during the use of OCs or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thrombophlebitis and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to-six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions:* There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.

4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, an increased risk was apparent between 6 and 12 months of use.

7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. Three studies have been performed with the Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alterations in lipid metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.

9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.

10. *Bleeding Irregularities:* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding:* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See "Warnings" section for information on the carcinogenic potential of OCs.

Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."

Nursing Mothers—See "Warnings."

Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted.: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is first taken later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information and package insert.

References: 1. Data submitted to FDA. **2.** Skouby SO, Kühl C, Mølsted-Pedersen L, et al: *Am J Obstet Gynecol* 1985;153:495-500. **3.** Vermeulen A, Thiery M: *Contraception* 1982;26:505-513.

BERLEX™
Dedicated to innovation in OB/GYN medicine

Berlex Laboratories, Inc., Wayne, New Jersey 07470

© 1986 Berlex Laboratories, Inc. All rights reserved.

430-27

American Journal of Obstetrics and Gynecology

Founded in 1920

volume 156 *number* 1 JANUARY 1987

CLINICAL SECTION

Clinical Opinion

Screening for postmenopausal osteoporosis

Margaret R. Davis, M.D.

Washington, D. C.

Postmenopausal osteoporosis is an area of increasing concern to the gynecologist. Fractures will develop in approximately 15% to 25% of women in their later years and preventive therapy is best aimed specifically at this group. Predicting who will be the ones to sustain fracture is a difficult and imprecise clinical task. The clinical, laboratory, and radiologic methods available for this purpose are reviewed. A rational approach to this problem in today's setting is outlined. (AM J OBSTET GYNECOL 1987;156:1-5.)

Key words: Osteoporosis, bone mineral content, photon absorptiometry, computed tomography

The clinical diagnosis of osteoporosis is made when an atraumatic fracture associated with qualitative or quantitative evidence of reduced bone mass is documented. Recently postmenopausal osteoporosis has become a burgeoning area of interest in terms of medical, social, and economic costs; it is also a problem that is appropriately coming under the purview of the gynecologist. Prevention of fracture is the primary goal of intervention. To routinely identify women at particular risk, one should have reasonable and cost-effective screening criteria that demonstrate the ability to predict the likelihood of future fracture.

This article will review for the clinician the clinical, laboratory and radiologic methods used in screening patients for the development of postmenopausal osteoporosis. A conclusion will be drawn regarding a rational approach that could be used to determine who might benefit from specific preventive measures.

History

The correlation of osteoporosis with the following factors is well documented: white and Oriental race, slender build and short stature, and the onset of early menopause (natural or surgical). A history of nontraumatic fractures in other female family members is significant.

The relationship of osteoporosis to dietary factors is less firmly established, but some dietary factors may be of importance by virtue of their effect on calcium metabolism and body weight. Poor calcium intake has been identified in a significantly greater percentage of patients with osteoporosis than in the general population. Diets high in caffeine, alcohol, phosphorus, animal protein, and some acidifying foods decrease calcium absorption or promote excretion.

Other life-style habits should be investigated. Cigarette smoking has been linked to earlier menopause. Bone loss is accelerated with immobilization and prolonged bed rest; thus sedentary living is an additional risk factor.

Table I lists some causes of secondary osteoporosis. Careful questioning for a history of those conditions or symptoms suggesting them, along with a drug history, is mandatory.

Even patients with established osteoporosis may offer no symptoms. Severe, local back pain may develop during routine activity, but usually subsides within several months. Persistent pain can occur from altered spinal mechanics, but many compression fractures cause no pain at all. The patient may report body shortening or a "dowager's hump." Severe kyphosis can promote pulmonary dysfunction, abdominal discomfort, early satiety, bloating, and constipation.

Progressive loss of alveolar bone leading to tooth loss

From the Department of Obstetrics and Gynecology, George Washington University.

Reprint requests: Margaret R. Davis, M.D., Department of Obstetrics and Gynecology, 2150 Pennsylvania Ave., N.W., Washington, DC 20037.

Table I. Causes of secondary osteoporosis

Hyperthyroidism
Hyperparathyroidism
Hypercortisolism
Hypogonadism
Renal dysfunction
Malabsorption disorders
Connective tissue disorders (such as osteogenesis imperfecta)
Neoplastic disease (such as multiple myeloma)
Drug use
Heparin
Corticosteroids
Anticonvulsants
Aluminum-containing antacids
Methotrexate

may be a manifestation of osteoporosis. An early need for dentures with frequent refittings and a history of periodontal disease should alert the clinician.

Physical examination

The only physical signs may be loss of height or the development of kyphosis, or both, with loss of lumbar lordosis. Signs of other diseases should be sought.

Simplified anthropometric measurements should be recorded, including accurate weight, standing height, and arm span. At bone maturity (approximately 30 years of age), arm span should equal head-to-heel height. Skin-fold measurements can be made with a skin-fold caliper at the subscapular, suprailiac, triceps, and calf sites; by using reference tables, an estimation of the percent of body fat, which does correlate with the development of osteoporosis, is derived. It is of interest that although osteoporosis and osteoarthritis are both common in the older age groups, they are rarely seen together; indeed, the anthropometric profile for each is quite separate, with differences in skin-fold thickness constituting the most important distinction.

Laboratory methods

No one laboratory method has been found to screen reliably for postmenopausal osteoporosis. In the uncomplicated case, complete blood count, sedimentation rate, serum protein electrophoresis, calcium, phosphorus, alkaline phosphatase and urinalysis values are all normal. These tests and others, however, are useful in identifying causes of secondary osteoporosis. For example, a low serum calcium level, a serum phosphorus level <3 mg/dl, or an elevated alkaline phosphatase level should prompt one to pursue the diagnosis of osteomalacia.

Calcitonin levels and reserve do not correlate with bone density.[1] Hydroxyproline excretion is similar in osteoporotic and nonosteoporotic groups of women. Several more specialized tests have been studied. Serum bone Gla protein is a specific marker for bone turnover but may be high, normal, or low in osteoporotic patients, reflecting the heterogeneity of histologic findings.[2] One study examined alkaline phosphatase isozymes and suggested that elevated bone isozyme levels in the perimenopausal period predisposed to the development of osteoporosis as reflected in higher fracture rates.[3]

Hormonal parameters have also been investigated. Most studies show no major differences in cortisol, androstenedione, estradiol, and estrone levels in osteoporotic and normal women. Johnston et al.[4] found that menopausal women who are "rapid bone losers" have very low circulating progesterone levels (<0.5 ng/ml), whereas "slow bone losers" have significantly higher levels. However, this is not a specific or highly discriminating test. Recently, the potential usefulness of a short-term gonadotropin-releasing hormone antagonist test has been investigated in primates. Its aim would be to prospectively predict an individual's risk of osteoporosis by assessing her vulnerability to negative calcium balance with estrogen deficiency.[5]

Two tests are commonly used in the early identification of clinical osteoporosis. An elevated fasting urinary calcium/creatinine ratio (>0.16) and an elevated fasting urinary hydroxyproline/creatinine ratio (>0.012) suggest rapid bone turnover with excess osteoclast activity. Again these are nonspecific parameters and there is substantial overlap in results from normal and osteoporotic groups of women.

Histomorphometry

Histomorphometry, usually performed on a bone biopsy specimen from the iliac crest, remains the most definitive invasive study for osteoporosis. Osteomalacia and certain forms of secondary osteoporosis can be excluded. Although the biopsy evaluates only one area of bone, it is a quantitative measurement and provides a reliable diagnostic indication of skeletal disease. The concept of histologic heterogeneity of postmenopausal osteoporosis has recently been developed on the basis of analyses of iliac crest bone biopsies. Although there is little morbidity in the technique, specialized equipment and expert analysis are required, and patient acceptance may be low. It can be reserved for a selected group of patients.

Radiologic methods

Standard radiographs. Before reduction of bone mass can be detected on standard films, 20% to 40% of bone mineral must be lost. Characteristic abnormalities on lateral spinal films include radiolucency of the vertebral spongy bone compared with the subchondral plates. Concave defects in the upper and lower surfaces of involved vertebra are noted, with

prominence of the vertical trabecular pattern. One or more vertebral crush fractures may be seen. X-ray evidence of osteoarthritis is usually lacking. In the peripheral skeleton there is diffuse demineralization with cortical thinning of the long bones. A standard panoramic dental radiograph may indicate severe residual alveolar ridge reduction. These methods are relatively crude and insensitive. If used as screening measures, a high degree of bone loss will already have occurred before preventive measures can be instituted.

Radiogrammetry. This is a standard x-ray technique that measures the thickness of the cortex of metacarpal or other tubular bones. It requires the ability to take reproducible bone radiographs and make fine-caliber measurements. The outer diameter of the bone is measured along with the marrow cavity diameter; subtraction gives the combined cortical thickness. Compared with those of a large, normal population, these measurements are usually quite precise, and this can be a good technique for cross-sectional and longitudinal studies. Because absolute bone mineral content and intracortical porosity are not measured, however, this method only provides information on relative changes in bone volume. Moreover, it is only applicable to the appendicular skeleton. Nonetheless, decreased cortical thickness is a measure of decreased bone mass, and spine and hip fractures are uncommon among women having a normal cortical thickness. The incidence of such fractures increases with decreasing cortical diameter.

Microradioscopy of the hand bone involves the aforementioned method with the use of fine-grain industrial film and increasing exposure time. With this procedure, cortical thickness and intracortical porosity can be measured separately, allowing assessment of endosteal and intracortical resorption. The linear endosteal resorption rate can be determined over a period of time; inasmuch as estrogen loss promotes bone resorption at that site, this simple procedure may be useful for detection of early developing osteopenia in perimenopausal women.[6]

Photodensitometry. Photodensitometry uses the bone mineral image on standard x-ray film as an indicator of photon absorption by bone and thus an indirect measurement of bone mineral content. The degree of whitening of the film is measured by a photodensitometer and compared with a simultaneous exposure of a calibrated bone mineral analogue. Bones with little overlying soft tissue, such as the radius, are best measured. Some researchers have found good correlation between radial bone mineral mass measured by this method and the partial body calcium mass measured by in vivo neutron activation, suggesting that changes in the proximal radius parallel those in the central skeleton. However, the technique does require expertise and is somewhat time-consuming. Precision (reproducibility) and accuracy is relatively poor.[6,7]

Singh index. The change in the trabecular pattern of the proximal femur is the basis of the Singh index. When bone mass is diminished, the first trabeculae to disappear are those subject to minor functional stresses. The disappearance of various groups of trabeculae seems to occur in a precise order, allowing the recognition of seven degrees of progressive osteopenia. A standard x-ray of the pelvis is taken, with the lower limbs slightly internally rotated to better view the proximal femur. Quantitative values for bone mineral content have been obtained by densitometric methods that correlate with each of Singh's categories. Although the Singh technique is easy to perform and interpret, the accuracy is poor, and prospective studies have concluded that the Singh index is of little prognostic value.[8]

Compton scattering. This is a largely experimental approach that measures trabecular bone density by using Compton scattered radiation. The extent of scattered radiation depends on the electron density (thus total density of bone plus marrow) of the scattering medium. The precision and accuracy of density determinations is about 3%.[9]

Single- and dual-photon densitometry. Single-photon absorptiometry is now probably the most widely used technique for estimation of bone mass. A highly collimated beam of monoenergetic radiation is passed across a limb, and the transmitted radiation is monitored with a scintillation detector. To ensure even soft tissue coverage, the limb is encased in a water bath or other tissue-equivalent medium. The amount of mineral in the bone is proportional to the change in intensity of the beam, and bone mass is calculated by comparing it with known standards. The most widely studied site is the radius. In an attempt to correlate more closely with the axial skeleton, a "5 mm site" has been established for measurements near the distal radius, which may be composed of more than 50% trabecular bone.[10] Precision (reproducibility) is good, 2% to 5% in the clinical setting. The principal determinant is repositioning at the same site on repeated scans. Because the distal radius is irregular in shape, whatever advantage is gained by measuring there, where more active trabecular bone is present, may be lost in reduced precision.

From repeated measurements of this kind, data have been computed, averaged, and printed out on a "fracture index chart." Serial measurements are plotted on the chart every 6 to 12 months, and the fracture risk is predicted. Specifically, bone density (mineral content/bone width) of 325 mg/cm^2 was found to be a "fracture threshold"; it is the distal radius density value >95% of all values from fracture patients.[11] Unfortunately, overlap among groups is significant, in the range of

Table II. Radiation exposure of various techniques

Technique	*Local exposure (mrem)*
Radiogrammetry	≤100
Photodensitometry	≤100
Compton scattering	300 - 500
Photon absorptiometry	
Single-energy	2 - 5
Dual-energy	5 - 15
Computerized tomography	
Single-energy	200 - 250
Dual-energy	250 - 400
Neutron-activation analysis	300 - 5000

15% to 20%. As might be expected, this technique does not consistently reflect changes occurring in other parts of the skeleton and other types of bone.

Attempts to apply single-photon absorptiometry to areas of trabecular bone have been only partially successful. Major problems include soft tissue interference and increased precision errors in measuring at many sites. The os calcis (calcaneus or "heel bone"), which is more readily accessible, has been measured by means of rectilinear scanning methods, necessary because of its irregular shape. The extent of os calcis demineralization seems to correlate better with the degree of spinal osteopenia and shows a consistent relationship to fracture prevalence. It has been postulated by some that this measurement is the best predictor of nonspine and spine fracture risk, short of actual density determinations of the site in question.[12]

Dual-photon absorptiometry makes measurements at two distinct energies, thereby eliminating the need to have constant thickness of soft tissue around the bone. Direct measurements of the femoral neck and spine are feasible. Like the single-photon method, however, it cannot discriminate between cortical and trabecular bone at these sites. The long-term precision is approximately 2% to 3%, and its greatest reliability is in following changes in skeletal bone mineral content over a period of time. Good discrimination is seen between subjects with fractures and control subjects; those with compression fractures average approximately 2 SD below control values but only 1 SD below normal in radius bone density. It has also been demonstrated that sequential changes occur much more rapidly in the lumbar spine than in the appendicular skeleton, one of the reasons that single-photon absorptiometry methods are relatively poor in predicting fracture risk.

Single- and dual-energy computerized tomography. The usefulness of computerized tomography for measurement of bone mineral is its ability to obtain a quantitative image in either the axial or the appendicular skeleton and separately measure trabecular or cortical bone. Recently, sophisticated modifications in standard computerized tomographic scanners, with the use of special software and calibration techniques, have specifically allowed determination of vertebral cancellous bone mineral content with a precision of 2% to 3%.[13] Excellent correlation has been found between vertebral trabecular mineral determined by computerized tomography and iliac crest trabecular bone volume determined by histomorphometry.[14] Individual patients with rapid bone loss can be identified in 12 to 24 months. The relationship of vertebral mineral content by computerized tomography to fracture risk is similar to other bone mass measurement techniques; that is, there is a threshold value above which fractures are unlikely and below which they may occur. It has been found that compression fractures or vertebral wedging was generally absent in patients with vertebral mineral values >110 mg/cm^3, whereas almost all patients with values <65 mg/cm^3 suffered fractures.[15]

Neutron activation. Neutron activation analysis is considered the "gold standard" for calculating body calcium content. By neutron irradiation the calcium 48 of the body is converted to calcium 49, and the subsequent decay back to calcium 48 is measured with a counter. Because calcium is a constant fraction of bone mineral, it can be interpreted as an indicator of skeletal mass. Any extraosseous calcification will detract from the method's validity. Both total body and local calcium content can be measured; however, this method that is limited to the spine, for instance, involves higher radiation doses with a precision of 3% to 5%. As with the other techniques, overlap is seen in results obtained from osteoporotic and normal populations.[16]

Comment

No single measurement, even the most perfect, will accurately discriminate between patients who will and will not have fractures. Other variables, including wide biologic variation, time, and stress, all factor in with bone density in determining the potential for osteoporosis. In considering the above methods for clinical practice, the information that can be derived from them and their limitations should be kept in mind. Options range from fairly gross measurements of integral (cortical plus trabecular) bone in peripheral sites to precise quantitation of trabecular bone in vertebral sites. Common sense would lead one to believe that the best techniques would be the ones that measure the actual areas of greatest clinical concern—the spine and the hip. Dual-photon absorptiometry and computerized tomograpic scanning are the two methods that meet these criteria for clinical utilization. They have low errors of precision and accuracy and have been shown repeatedly to be useful for diagnosis and for monitoring of changes.

In addition, other considerations are important. All

of the noninvasive methods for determining bone mass involve a radiation dose (see Table II). Practicality would dictate that only the standard radiographic methods and single-photon densitometry are easily adapted to routine screening in a private office; these are the least discriminating tests to use in predicting risk. Cost, of course, will also dictate what is practiced. Computerized tomographic scanners cost $750,000 to $1 million, with testing charges of $300 to $500. The cost of a dual-photon bone scanner ranges from $35,000 to $40,000, with scanning costing $125 to $200. A single-photon densitometer is priced less than $20,000. Third-party payments of these costs are variable at best. The Blue Cross–Blue Shield insurance plan presently recommends that both single- and dual-photon absorptiometry be considered "experimental/investigational" and therefore not covered. Local plans may or may not opt for this. In Florida, insurance covers single-photon absorptiometry if the diagnosis is osteoporosis but not if it is used as a screening tool. In New York, single-photon absorptiometry is reimbursable whereas the dual method is not.

The long-term efficacy of therapeutic methods is not definitely known, but it is estimated that prophylactic hormone replacement in postmenopausal women could reduce the hip fractures rate by 40% to 60%. The current diagnostic related group (DRG) allowance for treatment of hip fracture is approximately $9000. Yearly bone scans and endometrial biopsies for a woman from 50 to 80 years of age may approach that figure, but of course the number of women screened and treated will far exceed the number who sustain fracture.

With technology as it presently exists, most clinicians for most patients will probably continue to rely heavily on clinical data alone in evaluating for postmenopausal osteoporosis. Patients considered on a clinical basis to be at especially high risk may warrant referral for dual-photon absorptiometry or computerized tomographic scanning. These should be done annually at least through the 8 to 10 years immediately after menopause, when maximal bone loss is occurring. Third-party coverage should become standard. It is not to be presumed that hormone replacement therapy is warranted only in those who undergo scanning and are found to have decreased bone mineral content.

REFERENCES

1. Tiegs R, Body JJ, Wahner HW, et al. Calcitonin secretion in postmenopausal osteoporosis. N Engl J Med 1985; 312:1097.
2. Delmas PD, Wahner HW, Mann KG, Riggs BL. Assessment of bone turnover in postmenopausal osteoporosis by measurement of serum bone Gla-protein. J Lab Clin Med 1983;102:470.
3. Collins DP, Rimm A, Bowman K, Foley DV. Possible prediction of accelerated osteoporosis by alkaline phosphatase isoenzymes. AM J OBSTET GYNECOL 1984;149:304.
4. Johnston CC, Norton JA, Khairi RA, et al. Age-related bone loss. In: Barzel US, ed. Osteoporosis. New York: Grune & Stratton, 1978, vol 2:91.
5. Abbasi R, Hodgen GD. Predicting the predisposition to osteoporosis. JAMA 1986;255:1600.
6. Meema HE. Non-invasive measurements of bone loss in peripheral bones. Clin Invest Med 1982;5:157.
7. Meckelnburg RL. Mechanism and detection of osteoporosis. Del Med J 1985;57:355.
8. Cabitza P, Favini P, Verdoia C. Singh's method of the radiological quantitative evaluation of osteoporosis compared with the densitometric method. Ital J Orthop Traumatol 1982;4:479.
9. Mazess RB. Noninvasvie methods for quantitating trabecular bone. In: Avioli LV, ed. The osteoporotic syndrome. New York: Grune & Stratton, 1983:85.
10. Awbrey BJ, Jacobson PC, Grubb SA, et al. Bone density in women: a modified procedure for measurement of distal radial density. J Orthop Res 1984;2:314.
11. Grubb SA, Jacobson PC, Awbrey BJ, et al. Bone density in osteopenic women: a modified distal radius density measurement procedure to develop an "at risk" value for use in screening women. J Orthop Res 1984;2:322.
12. Wasnich RD, Ross PD, Heilbrun LK, Vogel JM. Prediction of postmenopausal fracture risk with use of bone mineral measurements. AM J OBSTET GYNECOL 1985;153:745.
13. Cann CE, Genant HK. Precise measurement of vertebral mineral content using computed tomography. J Comput Assist Tomogr 1980;4:493.
14. Genant HK, Ettinger B, Cann CE, et al. Osteoporosis: assessment by quantitative computed tomography. Orthop Clin North Am 1985;16:557.
15. Cann CE, Genant GK, Kolb FO, Ettinger B. Quantitative computed tomography for prediction of vertebral fracture risk. Bone 1985;6:1.
16. Harrison JE. In vivo neutron activation analysis. Clin Invest Med 1982;5:161.

Breech delivery: Why the dilemma?

Stephen A. Myers, D.O., and Norbert Gleicher, M.D.
Chicago, Illinois

A critical review of selected studies of breech delivery is presented with special attention to the statistical analysis of outcome for low birth weight and term breech delivery. Analysis of the data fails to support the routine use of cesarean birth for all cases of breech delivery except for those with hyperextension of the head. The implication of these findings is discussed. (Am J Obstet Gynecol 1986;155:6-10.)

Key words: Breech delivery, cesarean section

Cesarean delivery rates continue to increase in spite of National Institutes of Health consensus report recommendations to the contrary. The abdominal route of delivery is often chosen even when clear, convincing data fail to demonstrate any advantage over the vaginal route, with repeat cesarean sections representing the most significant example. Because breech delivery accounts for only 10% to 15% of all cesarean sections (3% of all deliveries) and because many authors advocate routine cesarean section for breech delivery, not much attention has been focused on this cesarean delivery indication. It is the purpose of this review to critically analyze the obstetric literature in order to answer the question of whether cesarean section for breech presentation results in better obstetric outcome when compared with vaginal delivery. An all-encompassing review of the breech literature is neither relevant to the above question nor within the scope of this communication. A more complete review of all breech data has recently been published.[1] Several key issues and their key references will, however, be discussed in more detail.

Review of published data

Outcome comparisons of breech delivery and delivery in cephalic presentation, which may vary in magnitude, are nonetheless uniform in their results. For most of the important end points, infants born in breech presentation do worse than those in cephalic presentation. This comparison has been summarized by Seeds and Cefalo[2] (Table I). With this adverse outcome in mind, increasing use of cesarean section for breech delivery has occurred on the basis of the assumption that abdominal delivery would improve outcome. Major factors to be considered in analysis of this issue are: frank versus nonfrank breech presentation, the use of Piper's forceps, preterm breech delivery, and term breech delivery.

Table I. Risks associated with breech presentation

Fetal risk	*Approximate incidence*
Prematurity	16%-33%
Major anomalies	6%-18%
Birth trauma	13 times normal risk
Cord prolapse	5-20 times normal risk
Intrapartum asphyxia	3-8 times normal risk
Spinal cord injuries and deflexion	21%
Hyperextension of head	5%
Arrest of aftercoming head	8.8%

Modified from Seeds and Cefalo.[2]

Frank versus nonfrank breech

The observation has been made that nonfrank breech presentation is accompanied by increased perinatal mortality.[3] This observation has been used to justify the suggestion that patients with infants with nonfrank breech presentation not be allowed to labor. However, two important variables that affect outcome compound the analysis of nonfrank breech presentations—birth weight and cord prolapse.

Goldenberg and Nelson[4] have demonstrated that the proportion of breech deliveries that are nonfrank increases as birth weight decreases. They reviewed the records of 141 patients with breech presentation who were delivered vaginally. Their findings reveal, in infants weighing <2000 gm, that 66% of all breech presentations are nonfrank, whereas only 33% are frank. Consequently, data comparing frank and nonfrank presentations alone may include differences in birth weight distribution that will also affect outcome and survival. A second factor may also compound outcome analysis comparing frank and nonfrank breech presentation. Nonfrank breech presentations are associated with significantly more instances of cord prolapse.[2]

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Mount Sinai Hospital Medical Center and Rush Medical College.
Supported by the Foundation of Reproductive Medicine, Chicago, IL 60610.
Reprint requests: Stephen A. Myers, D.O., Division of Maternal-Fetal Medicine, Mount Sinai Hospital Medical Center, California Ave. at 15th St., Chicago, IL 60608.

Table II. The low birth weight breech

Author	Years of study	Ratio of vaginal to cesarean deliveries	Survival ratio of vaginal to cesarean deliveries			Comments
			Birth weight			
			1000-1499 gm	1500-1999 gm	2000-2499 gm	
Woods[5]	1970-1975	61/16	35/75	0	0	Head entrapment ×7 in vaginal group. Respiratory distress syndrome cause of mortality in >50%
Karp et al.[3]	1970-1975	47/18	0	0	0	17% prolapsed cord in non-frank breech
Bowes et al.[8]	1970-1977	119/32	35/75	0	0	Includes 500-1500 gm birth weight since no difference with 500-999 gm documented
Duenhoelter et al.[9]	1972-1977	44/44	45/100	0	0	60% of deaths before 1974
Mann and Gallant[15]	—	279/157	0	0	0	No difference in 500-999 gm group. No difference in Apgar scores
Effer et al.[16]	1973-1980	67/95	0	0	0	No difference in 500-999 gm group
Gimovsky et al.[6]	1980	25/44	0/67	0	0	Similar for 500-999 gm group. Mortality only <1300 gm
Main et al.[11]	1977-1981	123/93	42/71	0	0	Includes 67 deliveries of 750-999 gm

Unless outcome analysis includes adjustments for these two potential biases, incorrect conclusions may follow.

Two studies that do not have the above biases have failed to demonstrate any difference in outcome for frank and nonfrank breech delivery. Woods,[5] in a retrospective review of 73 breech deliveries, failed to show any difference in outcome of frank or nonfrank breech delivery. He also failed to show any benefit of cesarean section for either group. Similarly, Gimovsky et al.,[6] in a prospective randomized study, failed to demonstrate a beneficial effect of cesarean section. In that study all infants with nonfrank breech presentation were randomized to either abdominal or vaginal delivery. The groups were comparable in birth weight and gestational age. No differences in the rate of mortality or trauma were demonstrated. In spite of these data, cesarean section continues to be recommended for nonfrank breech presentations.

Piper's forceps

Milner[7] has demonstrated that application of Piper's forceps to the aftercoming head will reduce neonatal mortality by one half in all birthweight groups. He observed a neonatal mortality of 162/1000 when Piper's forceps were not used, compared with 28/1000 when Piper's forceps were used. Moreover, this difference was still present when the data were analyzed by specific birth weight groups, for instance, for 1000 to 1499 gm birth weights, 22% mortality with forceps and 47% mortality without forceps.[7] Although these mortality figures are not directly comparable to more recent information, no published data refute Milner's conclusion. Despite this observation, reports of vaginal breech delivery continue without either discussion of the use of Piper's forceps or tabulation of frequency of their usage. It can only be stressed that the use of Piper's forceps be more routinely applied and reported.

Low birth weight breech

Perhaps the most controversial data regarding the effect of route of delivery on outcome are in the low birth weight breech group. Although fetopelvic disproportion does not occur, the possibility of head entrapment may be theoretically increased on the basis of the relatively larger head in a premature infant. Moreover, many authorities believe that premature infants are more susceptible to "trauma" during vaginal delivery. The eight most prominent studies covering this issue are summarized in Table II. Included in the table are the years in which the studies were performed, the total number of infants studied, and survivals in each of three birth weight groups. Inspection of these data reveals that no author was ever able to demonstrate a benefit of cesarean section in birth weights above 1500 gm, compatible with 30 to 31 weeks' gestational age, yet routine cesarean section generally is advocated for premature breech infants.

Very low birth weight breech

Data for the very low birth weight breech infant deserve further scrutiny. Studies of Woods,[5] Bowes et al.,[8] and Duenhoelter et al.[9] share a common flaw. In the early 1970s, the viability of infants weighing <1500 gm was clearly questioned. These three studies included

Table III. Mortality in very low birth weight breech deliveries (n = 37)

	Vaginal	*Cesarean section*	*Significance*
500-999 gm	12	9	
1000-1499 gm	3	13	
Mortality (%)	93	33	$p < 0.05$ (authors' data)
Sample <1000 gm (%)	80	41	$p < 0.05$ (our calculation)

Modified from Gimovsky et al.[6]

Table IV. Mortality in very low birth weight breech deliveries (n = 37)

	Vaginal	*Cesarean section*	*Significance*
750-999 gm	45/52	9/15	NS
1000-1249 gm	19/40	12/46	NS
1250-1499 gm	7/31	6/32	NS
No. of deaths/ Total No.	71/123 (58%)	27/93 (29%)	$p < 0.05$
Sample <1000 gm (%)	43	16	$p < 0.05$ (our calculation)

Modified from Main et al.[11]

patients who were delivered vaginally because they were thought in fact to carry previable infants. As an almost self-fulfilling prophecy, many of these infants subsequently died. Although cesarean section was occasionally carried out for maternal indications, it was usually only performed if the fetus was considered viable. Since the prognosis of viability was not prospectively controlled for in the management decisions of these three studies, the studies have to be considered of questionable validity and can be useful for comparative purposes only.

Two of the later studies (Table II) warrant further discussion. Gimovsky and Paul[10] reported a considerable benefit of cesarean sections in the very low birth weight group (Table III). However, 40% of deaths in their group delivered vaginally occurred in infants weighing <750 gm. Were these infants considered viable at Los Angeles County Hospital in 1980, or would they be considered as such even today? Moreover, the confounding effect of birth weight distribution differences between vaginal and abdominal groups was not considered. A significantly higher percentage of infants <1000 gm occurred in the vaginal group compared with the abdominal group (80% vaginal versus 41% abdominal; $p < 0.05$, our calculation). Surely the effect of birth weight on survival cannot be ignored. An identical error was made by Main et al.[11] (see Table IV).

Table V. Recommendations for breech delivery

Factors favoring vaginal delivery
- Birth weight <4200 gm
- Adequate pelvis
- Good progress of labor
- Absence of hyperextension of fetal head

Factors favoring cesarean section
- Birth weight >4200 gm
- Borderline pelvis
- Poor progress of labor (particularly descent) with adequate contractions
- Fetal distress
- Hyperextension of the fetal head

Although they correctly noted that there was no significant difference in survival in any of the three birth weight groups between 750 and 1500 gm, they chose to add the three weight groups together to come to the extraordinary conclusion that the vaginal delivery group did worse. In doing so, they failed to note that by lumping all three groups together, the vaginal group birth weights were skewed to those <1000 gm (43% under 1000 gm in the vaginal group versus 16% under 1000 gm in the cesarean group; $p < 0.05$, our calculation). Once again the confounding effect of birth weight on survival was interpreted as an effect of the route of delivery.

The most definitive data published to date on route of delivery for very low birth weight infants were recently presented by Kitchen et al.[12] from Melbourne. Their work included data on 326 infants born at 24 to 28 weeks' gestation between 1977 and 1982. Included in this group were 117 breech deliveries. The work of the Melbourne group stands out from all other published data for three major reasons. First, a prospective determination was made that all infants in the study were viable and consequently no infant's management was prejudiced by the expectation that the infant would die. Second, statistical analysis was performed with the use of a stepwise logistic regression analysis that took into account 14 different obstetric variables that could affect survival, including route of delivery. Last, and most important, half of the study infants were evaluated at 2 years of age with neurological and developmental assessments. We know of no other study of very low birth weight breech deliveries that includes these three crucial elements. The route of delivery was not prospectively randomized in the Melbourne study. It was assumed that vaginal delivery was safe and cesarean section was reserved for obstetric indications such as fetal distress and maternal disease. Only 16% of breech deliveries were by cesarean section. The authors found no difference between the two groups in either immediate or long-term outcome. Those advocating routine abdominal delivery for the very low birth weight

breech have no comparable data in support of their recommendation.

Term breech

Regarding term breech deliveries the two most significant studies for current obstetric practice are by Collea et al.[13] and Green et al.[14] Collea et al.[13] studied the outcomes of 208 patients with frank breech presentation at term in a prospective study with patients randomized to either vaginal or abdominal delivery. The conclusion of the authors was that vaginal delivery of the term frank breech was acceptable, provided patients fulfilled the minimum criteria of their study protocol.[13] There was no apparent advantage of cesarean section for the neonate. As expected, maternal morbidity was significantly greater in the abdominal delivery group.

A more provocative observation was made by Green et al.[14] in Montreal. When they retrospectively observed the outcome of 600 term breech deliveries during the period 1963 through 1973, they noted that birth trauma and birth asphyxia occurred in 18% of the deliveries. The cesarean section rate for that group was 22%. They reasoned that this incidence of birth trauma and birth asphyxia, which they considered unnecessarily high, could be reduced by the more liberal use of abdominal delivery. By 1978, they had been successful in increasing their cesarean section rate to 94% of all breech deliveries. Subsequent review of their data for 1978 and 1979 (n = 175) again indicated an incidence of birth trauma and birth asphyxia of 18%. This experience clearly demonstrates that the increase in cesarean section rate did not affect breech outcome.

The experience factor

It has been suggested that because today's obstetricians have inadequate experience with vaginal breech delivery, abdominal delivery represents a more prudent choice for "young practitioners." We reject this notion for several reasons. First, ignorance about the technique of breech delivery can result in trauma in both vaginal and abdominal delivery. A fetus will thus not benefit from abdominal delivery if the delivery technique used is inappropriate.

It is the profession's responsibility to provide training in breech delivery technique during the residency period. During this time a house officer has the opportunity to accumulate a concentrated, supervised experience, unmatched by any subsequent period of his or her professional life. The education of young physicians has to be the responsibility of senior obstetricians who have accumulated sufficient clinical experience in breech delivery to serve in a consulting capacity. Within such an educational framework, one could in fact foresee that these senior consultants will be available during vaginal breech deliveries at their institutions. Patients could thus be assured that breech deliveries are performed by the most experienced obstetrician in the safest way for mother and infant. After all, inexperience should never be the reason for a surgical solution. Experience is usually available.

Cost consideration

Cesarean section, compared with vaginal delivery, costs an additional $2700 per patient in hospital cost alone. Among 3.5 million deliveries per year in the United States, approximately 122,500 are in breech presentation. Currently, national cesarean section figures suggest that approximately 70% to 80% of all breech deliveries are performed by cesarean section. If the above-mentioned data from the Melbourne[14] and Montreal[16] groups are representative and a 20% cesarean section rate can safely be applied, 50% to 60% of breech deliveries (60,000 to 70,000 deliveries per year) are at present subjected to unnecessary cesarean sections. The annual national hospital cost for unnecessary cesarean sections for breech presentation thus approaches $200,000,000 per annum. These cost calculations do not include added costs for physician services, etc. Improved neonatal outcome could justify such cost. As should be evident, from the above-presented data, however, no such benefit exists.

Recommendation

On the basis of the review of obstetric literature, we suggest that previously held risk factors for vaginal delivery, such as nonfrank breech presentation, parity, and low or very low birth weight, are not associated with increased risk for birth trauma or birth asphyxia when vaginal delivery is compared with cesarean section. Patients presenting in labor with breech presentation must have the following: (1) ultrasound examination to exclude obvious anomalies, check placental localization, corroborate clinical estimate of fetal weight, and evaluate the angle between the cervical and thoracic spine; (2) clinical or x-ray pelvimetry if estimated fetal weight is >3500 gm or to corroborate hyperextension of the fetal head if ultrasound is unavailable or inadequate; (3) continuous electronic fetal monitoring; (4) adequate documentation of normal progress of labor. Should risk factors known to be associated with adverse outcome develop, cesarean delivery is to be used (see Table V). On the basis of the Montreal and Melbourne experience,[14, 16] the need for abdominal delivery should, however, not exceed 20% to 25% of all breech deliveries. Effective cesarean section monitoring programs for breech presentations therefore need to be developed. Such programs could include mandatory chart review and could be designed to tabulate which risk factors for vaginal breech delivery

have been documented on the chart. However, peer review can only be effective if a consensus develops in the professional's above-outlined guidelines. Most important, such an approach could be taken with confidence that no adverse effects on either mother or newborn infant would occur.

REFERENCES

1. Confino E, Gleicher N, Elrad H, Ismajovich B, David M. The breech dilemma. A review. Obstet Gynecol Surv 1985;40:330-6.
2. Seeds S, Cefalo R. Breech delivery. Clin Obstet Gynecol 1985;25:145-56.
3. Karp LE, Doney JR, McCarthy T, Meis PJ, Hall M. The premature breech: trial of labor or cesarean section? Obstet Gynecol 1979;53:88-92.
4. Goldenberg RL, Nelson KG. The premature breech. Am J Obstet Gynecol 1977;127:240-4.
5. Woods JR. Effect of low birth weight breech delivery on neonatal mortality. Obstet Gynecol 1979;53:735-40.
6. Gimovsky ML, Wallace RL, Shifrin BS, et al. Randomized management of the nonfrank breech presentation at term: a preliminary report. Am J Obstet Gynecol 1983;146:34-40.
7. Milner RDG. Neonatal mortality of breech deliveries with and without forceps to the aftercoming head. Br J Obstet Gynaecol 1975;82:783-5.
8. Bowes WA Jr, Taylor ES, O'Brien M, Bowes C. Breech delivery: evaluation of the method of delivery on perinatal results and maternal morbidity. Am J Obstet Gynecol 1979;135:965-70.
9. Duenhoelter JH, Wells E, Reisch JS, et al. A paired controlled study of vaginal and abdominal delivery of the low birth weight breech fetus. Obstet Gynecol 1979;54:310-3.
10. Gimovsky ML, Paul RH. Singleton breech presentation in labor: experience in 1980. Am J Obstet Gynecol 1982;143:733-9.
11. Main DM, Main EK, Maurer MM. Cesarean section versus vaginal delivery for the breech fetus weighing less than 1500 grams. Am J Obstet Gynecol 1983;146:580-4.
12. Kitchen W, Ford GW, Doyle LW, et al. Cesarean section or vaginal delivery at 24 to 28 weeks' gestation: comparison of survival and neonatal and two-year morbidity. Obstet Gynecol 1985;66:149-57.
13. Collea JV, Chein C, Quilligan EJ. The randomized management of term frank breech presentation. A study of 208 cases. Am J Obstet Gynecol 1980;137:235-42.
14. Green JE, McLean F, Paul Smith L, et al. Has an increased cesarean section rate for term breech deliveries reduced the incidence of birth asphyxia, trauma, and death? Am J Obstet Gynecol 1982;142:643-8.
15. Mann L, Gallant JM. Modern management of the breech delivery. Am J Obstet Gynecol 1979;134:611-4.
16. Effer B, Saigal S, Rand C, et al. Effect of delivery method on outcomes in the very low–birth weight breech infant: is the improved survival related to cesarean section or other perinatal care maneuvers? Am J Obstet Gynecol 1983;145:123-8.

Bound volumes available to subscribers

Bound volumes of the American Journal of Obstetrics and Gynecology are available to subscribers (only) for the 1987 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 156 (January-June) and Vol. 157 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the Journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular Journal subscription.

Immunoproteins in the endometrium: Clinical correlates of the presence of complement fractions C3 and C4

Delphine Bartosik, M.D., Ivan Damjanov, M.D., Ph.D., Richard R. Viscarello, M.D., and Judith A. Riley, M.D.

Philadelphia, Pennsylvania

The presence of complement fractions C3 and C4 in endometrial tissue was studied in a consecutive series of patients undergoing diagnostic laparoscopy, to determine their specific association with endometriosis. The incidence of complement in eutopic endometrium of patients grouped according to four diagnoses was: endometriosis, 66% positive (23 of 35); active pelvic inflammatory disease, 85% positive (11 of 13); combined endometriosis with pelvic inflammatory disease, 25% positive (one of four); laparoscopically normal pelvis, 67% positive (10 of 15). These differences were not statistically significant. Complement was equally likely to be found in proliferative, secretory, menstrual, or inflammatory endometrium. Endometrial complement was found less frequently in patients with severe endometriosis as compared with those with the mild form. Among patients with endometriosis and infertility, complement was much more likely to be found in patients with primary infertility than in those with secondary infertility ($p < 0.007$). In short-term follow-up of these patients, the absence of complement in the eutopic endometrium appeared to be a good predictor of subsequent pregnancies. (Am J Obstet Gynecol 1987;156:11-5.)

Key words: Endometriosis, pelvic inflammatory disease, complement, immunoglobulins, endometrium, infertility, autoimmune phenomena

Weed and Arquembourg[1] reported that the complement fraction C3 was found in the eutopic (uterine) endometrium of patients with endometriosis but was absent from control subjects. On the basis of these observations, they formulated the hypothesis that endometriosis is an autoimmune disease. However, no other studies of the specificity of this phenomenon have been reported. Therefore we studied a consecutive series of patients for the presence of complement fractions C3 and C4 in the uterine endometrium. Our observations are the subject of this report.[2]

Material and methods

Patient population. All patients in whom laparoscopy with endometrial biopsy was performed during a 4-month period were included in this study. There were a total of 67 patients. Clinical indications for performing laparoscopy are summarized in Table I. Thirty-two patients had either primay or secondary infertility and 47 patients had pelvic pain. There were 11 other pa-

Table I. Indications for laparoscopy among the patient population studied

	*Pelvic pain**	*Without pelvic pain†*	*Total*
Primary infertility‡	13	1	14
Secondary infertility§	10	8	18
Without infertility‖	24	11	35
Total	47	20	67

*The major pain manifestation in these patients was secondary dysmenorrhea. However, five patients had constant pelvic pain.

†There was no pelvic discomfort.

‡Defined as the inability to conceive after 12 months of unprotected intercourse but with no previous pregnancy.

§Defined as the inability to conceive after 12 months of unprotected intercourse; however, there had been an earlier pregnancy.

‖Fertility was not an issue in these patients. However, some of them were having unprotected intercourse.

From the Division of Reproductive Medicine, Department of Obstetrics and Gynecology, and the Department of Pathology, Hahnemann University.

Received for publication February 18, 1986; revised June 20, 1986; accepted July 11, 1986.

Reprint requests: Delphine Bartosik, M.D., Department of Obstetrics and Gynecology, Division of Reproductive Medicine, Hahnemann University, Broad and Vine, Philadelphia, PA 19102.

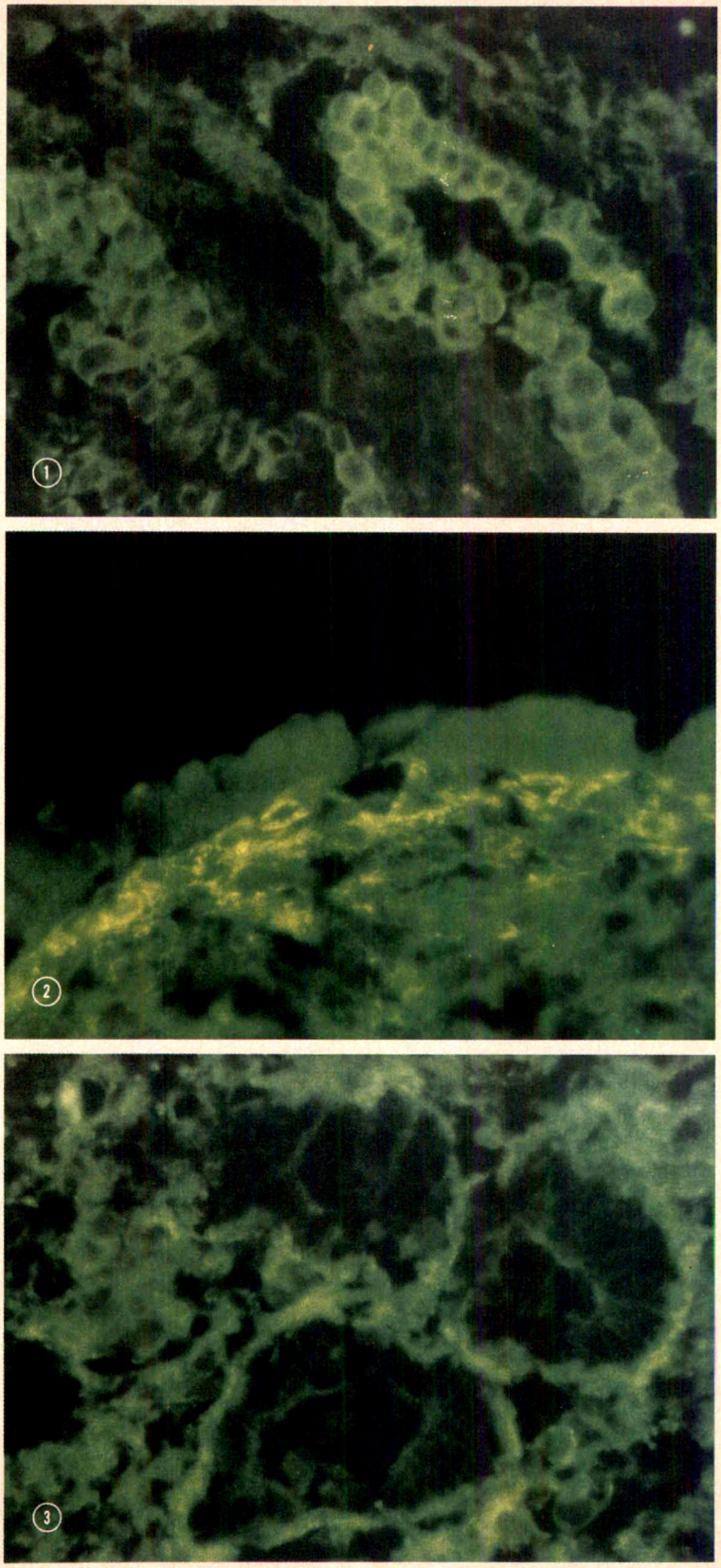


Fig. 1. Complement fraction C3 in the glandular epithelium of the endometrium.

Fig. 2. Complement fraction C3 under the surface of the endometrial lining.

Fig. 3. Complement fraction C3 around the glandular basement membrane and in the stroma of the endometrium.

Table II. Association between laparoscopic diagnosis and the presence of complement C_3 and C_4 in the endometrium

Presence (+) or absence (−) of C3 and C4 in the endometrium	*Endometriosis**				*All cases of endometriosis*	*Pelvic inflammatory disease*	*Neither endometriosis nor pelvic inflammatory disease*	*Both endometriosis and pelvic inflammatory disease†*
	Stage I	*Stage II*	*Stage III*	*Stage IV*				
$(-)C_3$, $(-)C_4$	4	5	3	–	12	2	5	3
$(+)C_3$, $(+)C_4C_4$	8	2	1	–	11	4	3	–
$(-)C_3$, $(+)C_4$	3	1	–	1	5	2	3	–
$(+)C_3$, $(-)C_4$	4	2	1	–	7	5	4	1
Total	19	10	5	1	35	13	15	4
Percent positive for C_3 and/or C_4	79	50	40	100	66	85	67	25

*Endometriosis is classified according to the staging proposed by The American Fertility Society.

†In all patients noted to have endometriosis and pelvic inflammatory disease, the former was stage I.

tients in whom the indications for laparoscopy were: evaluation of a pelvic mass (seven), laparoscopic tubal sterilization (two), and evaluation for tubal reanastomosis (two). Laparoscopy was performed in the standard manner with a second puncture probe used to permit visualization of all pelvic peritoneal surfaces.

Immunofluorescence microscopy.[3] The endometrial tissue was placed in sodium chloride, 0.15 mol/L transferred to the laboratory, frozen in liquid nitrogen, and embedded in OCT (Lab Tek, Naperville, Indiana). Sections of 8 μm were cut on a cryostat, postfixed in cold (4° C) acetone for 10 minutes, rehydrated, and processed further for incubation with the antibody. All incubations were performed in a moist chamber at room temperature for 1 hour. The fluorescein isothiocyanate–labeled antisera raised in goat or rabbit to human complement fractions C3 and C4 were purchased from Cappel Laboratories, Cochranville, Pennsylvania. Ratios of fluorescein to protein varied from 1.6 to 3.9. Monospecificity of the antisera was determined by immunoelectrophoresis and by Ouchterlony double diffusion. The optimal dilution for direct immunofluorescence staining was determined by titration on an open renal biopsy specimen known to contain deposits of immunoglobulin and complement and varied, in general, from 1 : 10 to 1 : 30. The same renal tissue was used as a positive control run in parallel in all endometrial specimens. The controls included tissue sections incubated with the same antibodies, but not labeled with fluorescein isothiocyanate or preabsorbed with human complement or simply with fresh human plasma. The preabsorbed antiserum was found to be unreactive with the "positive control," the kidney biopsy specimen as described above. Coverslips were applied, and the slides were mounted with glycerol and examined with a Leitz microscope outfitted with ultraviolet epiillumination.

The findings were subjectively evaluated and graded either as negative or as mild, moderate, or strongly positive (0 to 3). The reactivity of the uterine glandular epithelium, stroma, and vessels were evaluated separately. The diagnoses were not available to the pathologist when the slides were evaluated.

Routine histologic evaluation. Sections of the remaining endometrial tissue were fixed in 10% formaldehyde and stained with hematoxylin and eosin.

Statistical analyses. All comparisons of the prevalence of complement within the several diagnostic categories were analyzed by χ^2 tests.

Results

Immunofluorescence microscopy. Examples of endometrial tissue showing the presence of C3 are illustrated in Figs. 1 to 3. Fig. 1 illustrates C3 in the glandular epithelium of the endometrium. Fig. 2 illustrates C3 directly under the surface of the endometrial lining. Fig. 3 illustrates C3 around the glandular basement membranes and in the stroma. Microscopy of C4 immunofluorescence in the endometrium was similar to the figures presented for C3.

We were unable to determine a disease-specific pattern of C3 or C4 within the morphologic subsets of endometrium, that is, glands, basement membranes, or stroma. Subsequent analyses were therefore tabulated either as positive or as negative.

Incidence of complement in the uterine endometrium. Sixty-seven percent of all patients had either C3 or C4 in the uterine endometrium. With analysis according to laparoscopic diagnosis, 66% (25 of 35) of patients with endometriosis, 85% (11 of 13) of patients with pelvic inflammatory disease, 25% (one of four) of patients who had both endometriosis and pelvic inflammatory disease, and 67% (10 of 15) of patients with a laparoscopically normal pelvis had either C3 or C4 in the uterine endometrium. These data are presented in Table II.

Association between the presence of complement and the stage of endometriosis. Patients with endometriosis had staging done according to the criteria

Table III. Association between endometrial histologic features and the presence of complement C3 and C4 in the uterine endometrium

Presence (+) or absence (−) of C3 and C4 in the endometrium	*Proliferative*	*Secretory*	*Endometritis*	*Menstrual*
(−)C3, (−)C4	6	5	2	1
(+)C3, (+)C4	5	8	1	1
(−)C3, (+)C4	3	1	2	2
(+)C3, (−)C4	9	4	1	–
Total	23	18	6	4
Percent positive for C3 and/or C4	74	72	67	75

Table IV. Association between infertility and the presence of complement C3 and C4 in the endometrium

Presence (+) or absence (−) of C3 and C4 in the endometrium	*Primary infertility*	*Secondary infertility*	*Without infertility*
(−)C3, (−)C4	2	7	14
(+)C3, (+)C4	5	4	10
(−)C3, (+)C4	2	3	5
(+)C3, (−)C4	5	5	6
Total	14	19	35
Percent positive for C3 and/or C4	86	63	60

established by the American Fertility Society.[4] Among patients with endometriosis, there appeared to be a negative correlation between the stage of endometriosis and the incidence of complement. Thus 79% (15 of 19) of patients with Stage I, 50% (five of 10) of patients with Stage II, and 40% (three of five) of patients with Stage III endometriosis had demonstrable complement in the uterine endometrium. However, these differences were not statistically significant.

Association between endometrial histologic features and the presence of complement. The association between endometrial histologic features and the presence of C3 and/or C4 in the uterine endometrium is presented in Table III. Results of classical endometrial histologic features were not available in all patients, because if the amount of tissue obtained at biopsy was small, most of the tissue was subjected to immunologic analysis. We observed C3 and/or C4 in 17 of 23 samples (74%) of uterine endometrium in the proliferative phase, 13 of 18 samples (72%) in the secretory phase, three of four samples (75%) in the menstrual phase, and four of six samples (67%) with endometritis. Thus there does not appear to be any difference in the likelihood of the occurrence of C3 or C4 in the uterine endometrium obtained from different hormonal or inflammatory environments.

Association between infertility and the presence of complement. Among all patients, regardless of diagnosis, endometrial complement was slightly more likely to be found in patients with primary infertility. These data are summarized in Table IV. However, among the patients with endometriosis alone, endometrial C3-C4 was much more likely to be found in the group of patients with primary infertility than in those with secondary infertility. Ten of the 11 patients with endometriosis and primary infertility had endometrial complement, whereas only five of the 10 patients with secondary infertility had endometrial complement. These differences were statistically significant ($p < 0.007$).

In short-term follow-up of this group of patients, four patients with Stage I endometriosis became pregnant. None had endometrial complement.[2] Among all patients with Stage I endometriosis, 79% had endometrial complement.

Comment

The purpose of this study was to determine whether complement (C3 or C4) could be found in the eutopic (uterine) endometrium of patients with endometriosis and be absent from the endometrium of other patients. In contrast to the report of Weed and Arquembourg,[1] we observed that complement in the eutopic endometrium is not specific for patients with endometriosis. Endometrial complement was found among patients with pelvic inflammatory disease and in patients with a laparoscopically normal pelvis. For the purposes of this report, a laparoscopically normal pelvis is defined as one with neither endometriosis nor pelvic inflammatory disease. However, this group of subjects included a disproportionate number of patients with infertility and/or pelvic pain. Among the latter, most of the women either were not sexually active or were using barrier methods of contraception. Therefore, one cannot judge their fertility potential. Future studies must include a larger group of normal women, of established fertility (delivery within the previous year), who are requesting tubal sterilization. This would allow us both to inspect for the presence of endometriosis and to perform endometrial biopsies to quantify endometrial complement.

In two well-established in vitro fertilization pro-

grams, there were diametrically opposite results in terms of pregnancies among patients with endometriosis: The Norfolk group[5] showed high pregnancy rates, similar to those of their patients with tubal disease, while the British workers[6] showed significantly reduced pregnancy rates. The discrepancy may be due to the fact that most patients in the Norfolk group had undergone treatment for endometriosis while most of the British patients had received no therapy. Wild and Shivers[7] observed endometrial autoantibodies in the sera of 82% of patients with infertility and endometriosis. Although the presence of endometrial complement per se is nonspecific, it may be that complement-fixed endometrial autoantibodies are causally related to the infertility of some endometriosis patients. Such complement-fixing autoantibodies could induce a cytolytic, antiimplantation effect in the endometrium. This hypothesis would explain our observation that, in short-term follow-up, none of the patients with Stage I endometriosis who became pregnant had endometrial complement.

We also observed, among patients with endometriosis, that endometrial complement was more prevalent in patients with primary infertility than in those with secondary infertility. Possible explanations include a higher incidence of complement-fixing autoantibodies or a higher incidence of nonspecific endometrial complement in the primary infertility group. Whatever the explanation, it would seem prudent to view such patients (that is, primary versus secondary infertility) as separate subgroups of the total population of endometriosis-infertility patients.

In conclusion, although the presence of endometrial complement is a nonspecific finding, the data presented and reviewed support the hypothesis of Weed and Arquembourg[1] that endometriosis is an autoimmune disease. However, considerably more research must be done to confirm, extend, and interpret this hypothesis.[8]

REFERENCES

1. Weed JC, Arquembourg PC. Endometriosis: can it produce an autoimmune response resulting in infertility? Clin Obstet Gynecol 1980;23:885-93.
2. Bartosik D, Viscarello RR, Damjanov I. Endometriosis as an autoimmune disease [Abstract]. Fertil Steril 1984; 41(suppl):21KS.
3. Damjanov I, Fox N, Knowles BB, Solter D, Lange PH, Fraley EE. Immunohistochemical localization of murine stage-specific embryonic antigens in human testicular germ cell tumors. Am J Pathol 1982;108:225-30.
4. American Fertility Society: Classification of endometriosis. Fertil Steril 1979;32:633-4.
5. Chillik CF, Acosta AA, Garcia JE, et al. The role of in vitro fertilization in infertile patients with endometriosis. Fertil Steril 1985;44:56-61.
6. Yovich JL, Yovich JM, Tuvik AI, Matson PL, Willcox DL. In-vitro fertilisation for endometriosis. Lancet 1985;2:552.
7. Wild RA, Shivers CA. Antiendometrial antibodies in patients with endometriosis. Am J Reprod Immunol Microbiol 1985;8:84-6.
8. Bartosik D. Immunologic aspects of endometriosis. Semin Reprod Endocrinol 1985;3:329-37.

Labial fusion in prepubescent girls: A marker for sexual abuse?

Carol D. Berkowitz, M.D., Sandra L. Elvik, R.N., B.S.N., C.P.N.P., and Mary K. Logan, R.N., B.S.N., P.H.N.

Torrance, California

Review of more than 500 medical records of patients referred for sexual abuse evaluations revealed 10 cases of labial fusion. Patients ranged in age from 2 months to 5 years, and the duration of the fusion ranged from 2 weeks to 2½ years. No child had vulvovaginitis, dermatitis, or known genital trauma (for instance, straddle injury). Urinary tract problems were present in three patients. History and/or physical findings were consistent with sexual abuse in six of the 10 patients. Anal findings were grossly abnormal and consistent with anal penetration in all patients with a positive finding on examination. Therapy with conjugated estrogen cream was instituted in nine patients, with resolution of symptoms occurring in six. The exact cause of labial fusion is unclear. Fusion secondary to fecal soiling may occur in the young infant. Older girls may develop fusion after trauma such as that associated with sexual abuse, particularly vulvar coitus. (AM J OBSTET GYNECOL 1987;156:16-20.)

Key words: Labial fusion, vulvar fusion, labial agglutination, sexual abuse, child abuse

Labial fusion is defined as either partial or complete adherence of the labia minora.[1] Other names for this condition include vulvar fusion, synechiae of the vulva, adhesions of the labia minora, and agglutination of the labia minora.[2] The disorder occurs in prepubescent girls and has been reported in postmenopausal women (senile adherent vaginitis).[3] While the literature states that labial fusion is a frequent occurrence, the prevalence of the condition in the pediatric population is uncertain. Huffman reports the greatest incidence in girls 2 to 6 years old, while others note the highest frequency in infants under 2 years of age.[1, 4-6] Ben-Ami et al.[1] found in a prospective study that the condition was not present at birth but subsequently developed in nine of 1500 newborn infants followed up for 3 years, an incidence of less than 1%.

We recently began evaluating large numbers of children believed to have been sexually abused. During the course of these assessments, we became aware of a higher frequency of labial fusion than we had previously noted. In addition the patients seemed older than the infants with labial fusion we had previously encountered. We reviewed cases of sexual abuse retrospectively and describe our experience with labial fusion in a population of prepubescent girls undergoing evaluation for sexual abuse.

From the Department of Pediatrics, University of California, Los Angeles School of Medicine, Harbor/University of California, Los Angeles Medical Center.

Presented at the Ambulatory Pediatric Association Regional Meeting, Carmel, California, February 3, 1986.

Received for publication June 26, 1986; revised August 12, 1986; accepted August 14, 1986.

Reprint requests: Carol D. Berkowitz, M.D., Harbor/UCLA Medical Center, 1000 West Carson St., Bin 437, Torrance, CA 90509.

Methods and material

All patients were evaluated in the Pediatric Clinic of Harbor/University of California Los Angeles Medical Center in Torrance, California, a large, county-funded teaching hospital affiliated with the University of California Los Angeles School of Medicine. There are approximately 40,000 outpatient visits per year with 60 to 80 children seen per month for suspected sexual misuse. Patients are referred from numerous agencies, including law enforcement, protective services, juvenile court, community pediatricians, and psychologists.

Charts of all patients referred for sexual abuse evaluations between January and October 1985 were reviewed retrospectively to detect patients with labial fusion. The diagnosis of labial fusion was made on clinical grounds, on the basis of a thin line of adherence between the labia minora.[4] Patients were examined with simple visual inspection and with a colposcope by one or two of the authors. Records of patients with labial fusion were studied in detail, and the following information was noted: patient's age, degree of labial fusion, duration of labial fusion (if known), history and physical findings consistent with sexual misuse, history of trauma, urinary tract infections, or masturbation, other physical findings (particularly evidence of seborrheic or atopic dermatitis), therapeutic interventions, and follow-up evaluation.

Table I. Characteristics of patients with labial fusion

Case No.	*Age*	*Fusion (%)*	*History of sexual abuse*	*Results of physical examination**	*Urinary findings*	*Treatment†*	*Outcome*
1	5 yr	Complete	+	Hymen, normal Anal, abnormal	0	+	Partial resolution
2	4 yr	Partial	+	Hymen, not visualized Anal, abnormal	+	+	Partial resolution
3	5 yr	Complete	+	Hymen, normal Anal, abnormal	0	+	Partial resolution
4	4 yr	Complete	+	Hymen, not visualized Anal, abnormal	0	+	No follow-up
5	2 yr	Complete	+	Hymen, normal Anal, normal	0	+	Complete resolution Breast hypertrophy
6	4 yr	Partial	−	Hymen, abnormal Anal, abnormal	+	+	Resolution
7	9 mo	Complete	−	Hymen, not visualized Anal, normal	0	−	No resolution
8	13 mo	Complete	−	Hymen, not visualized Anal, normal	0	+	No resolution Breast hypertrophy
9	6 mo	Partial	−	Hymen, normal Anal, normal	0	+	Complete resolution
10	12 mo	Complete	−	Hymen, not visualized Anal, normal	+	+	No follow-up

*Physical findings consistent with sexual abuse.
†Treatment with conjugated estrogen cream: + = given; − = not given.

Results

Five hundred charts were reviewed. Seventy-five percent of the patients were female. Ten cases of labial fusion were detected, representing an incidence of about 3%. Table I summarizes these cases.

Patients ranged in age from 2 months to 5 years. Complete labial fusion with only a small anterior aperture was noted in seven (70%) and partial fusion involving only the posterior portion in three (30%) (Fig. 1). The duration of fusion could be estimated in five (50%) patients and ranged from 2 weeks to 2½ years. No patient had a specific history of trauma (for instance, straddle injury) other than related to sexual misuse. A history of masturbation was noted in one (Case 1). Masturbation was reported to occur on a daily basis and consisted of vigorous genital rubbing. Urinary tract symptoms were reported in three children. Two patients (Cases 2 and 4) had a history of urinary incontinence, and urinary reflux into the vagina was noted during voiding. One patient, 12 months old, had a documented urinary tract infection with *Enterobacter cloacae* that responded to ampicillin. No patient had a history of recurrent urinary tract infections.

Laboratory studies (urinalysis, urine culture, rectal and oral culture for *Neisseria gonorrhoeae*) were negative in all patients studied except for the child with the urinary tract infection. Two children (Cases 1 and 5) had vaginal cultures for chlamydia, and these were negative. No child had cultures taken for *Gardnerella*. The physical examination, apart from the genital and anal findings, was normal in all patients. No patient had physical evidence of seborrheic or atopic dermatitis.

Findings consistent with sexual abuse were noted in six patients. Four patients had both a history and a physical examination consistent with sexual abuse. One patient (Case 5) gave a history of sexual abuse, but there were no physical findings. In each case, the history was obtained from the accompanying caretaker or from the patient after extensive interviews. Physical findings consistent with sexual abuse were observed in five patients, including one patient who gave a negative history. Anal findings were grossly abnormal and consistent with anal penetration in all patients with a positive examination. Abnormal anal findings included lax anal tone in five (Cases 1, 2, 3, 4, and 6), transections of the external anal sphincter in two (Cases 3 and 4), and perianal tag in one (Case 2). The hymenal area was adequately visualized in six children and was abnormal in only one (Case 4). Examination of this girl showed an enlarged introitus with absent hymenal remnant. All four patients with no findings of sexual abuse were under 14 months of age.

Nine patients (90%) were treated with conjugated estrogen cream. Resolution of the labial fusion occurred in six. Two patients did not return for follow-up. Three patients experienced breast hypertrophy, which led to cessation of therapy. Other side effects of the estrogen included labial pigmentation and the appearance of fine, downy labial hair. Re-fusion after cessation of therapy was noted in one patient who was followed up for a year. This was the child who exhibited

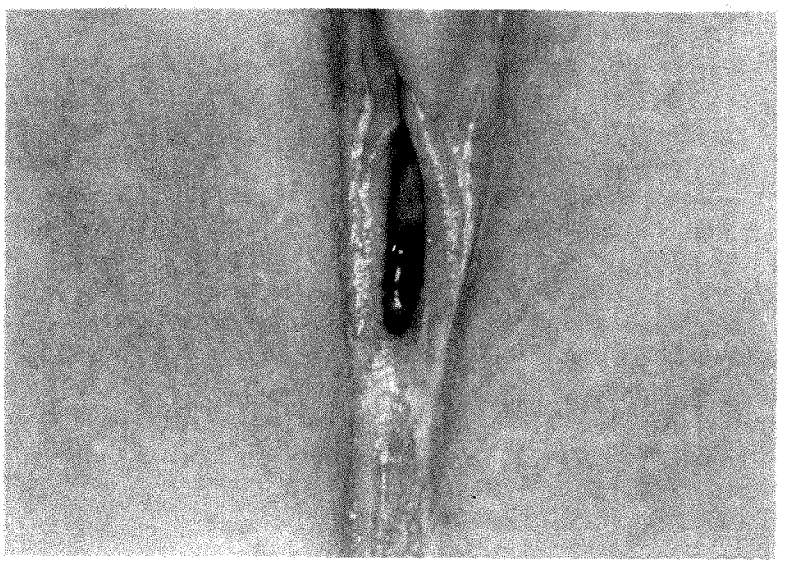


Fig. 1. Partial fusion of the labia minora in a 4-year-old Hispanic girl (Case 6).

daily masturbation. No patient had spontaneous resolution of the labial fusion during the study period.

Comment

Although the exact cause of labial fusion is unknown, a variety of disorders have been associated with the development of this condition. In the neonatal period, it may be seen with anatomic abnormalities, such as congenital absence of the vagina or ambiguous genitalia.[7] Ambiguous genitalia may indicate significant disease processes, such as true hermaphroditism, pseudohermaphroditism, or congenital adrenal hyperplasia. Labial fusion is an incorrect term in these conditions because labioscrotal folds are present. Exposure to excessive androgens results in failure of separation of the tissues of the perineal body between the urogenital sinus and the anus. Androgenic stimulation in the female fetus between the ninth and thirteenth weeks of gestation results in posterior adhesion of the labia along with clitoromegaly; after the thirteenth to fourteenth weeks of gestation, androgen exposure produces clitoromegaly alone. Labial fusion without clitoromegaly has been reported infrequently with congenital adrenal hyperplasia.[8]

Because fusion should be distinguished from failure of separation, Nowlin et al.[4] qualify the definition of labial fusion as "acquired post-inflammatory cohesion of the labia minora." The underlying mechanism is the denuding of the upper squamous epithelial layer of the labial mucosa with subsequent formation of a connective tissue bridge between the healing labia. Normally low estrogen levels in the prepubescent girl predispose to labial adhesions.[4-6] Adequate endogenous estrogens change the vaginal epithelium from a thin atrophic lining to a thick one containing glycogen.[3] In addition the neutral pH of the vaginal secretions in the prepubescent girl predisposes to inflammation and infection.[6]

Vulvovaginitis has been implicated in the formation of adhesions, although this relationship has not been systematically studied. Accurate vaginal cultures are difficult to obtain in the face of labial fusion, and there is usually no leukorrhea.[2] There are multiple causes of vulvovaginitis. Most cases are secondary to "poor hygiene," such as improper wiping techniques and playing in dirty sandboxes.[6] In the infant, feces and urine in the perineal area, as well as occlusive diapers, may inflame the labial mucosa and lead to fusion.[5] Nowlin et al.[4] reported no cases of fusion secondary to gonococcal infection or "frank nonspecific vulvitis." Likewise, Bowles and Childs[2] had no cases in which there was a history of vulvitis, vaginitis, trauma, or severe injury. Nonspecific vaginitis has recently been reported with *Gardnerella*.[9] Neither Nowlin et al. nor we performed cultures for *Gardnerella*. No child in our series had an antecedent history of vulvitis, and cultures for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* were negative. Vakar,[10] on the other hand, reported vulvovaginitis in 47% of his cases, although some of these had "physiologic desquamative vulvovaginitis" of the newborn infant. In this condition the neonatal mucosa is felt to be particularly vulnerable to invasion by stool flora.

Children with seborrheic dermatitis may experience recurrent vulvitis.[6] Such children are particularly prone to candidiasis. Atopic eczema infrequently leads to inflammation of the vulva. No patient in our series had evidence of either of these conditions, and fusion sec-

ondary to atopic or seborrheic dermatitis is reported in a minority of patients.[6]

A wide assortment of acute infectious illnesses has been implicated. These include cholera, diphtheria, scarlet fever, pneumonia, and typhoid fever.[11] Measles and chickenpox may produce lesions in the vulva and vagina with subsequent fusion.[6] No patient in our series had any of these conditions.

The role of trauma in the development of labial fusion has not been previously explored. Taylor[12] reported the case of a child with labial fusion after a "picket fence injury." No child in our series had a history of a straddle or picket fence injury. One would not expect such injuries to traumatize the labia minora.[2] The mechanism of a straddle injury is such that the labia majora and/or periurethral structures are crushed between the pelvic bone and an external object. Vigorous masturbation may lead to denuding of the labial mucosa and predispose to fusion. Self-stimulation is usually around the clitoris since this is the area of greatest sensation. Diffuse rubbing around the labia minora may occur with autostimulation or vulvar coitus. The latter is a form of sexual activity in which the penis is rubbed back and forth between the labia. It is possible that fusion may occur after such frictional irritation.

Eliciting a history of sexual abuse in the prepubescent child may be difficult. Children are often reluctant to disclose such information. They may say that the molestation happened to someone else. Interviews are facilitated by the use of anatomically correct dolls, puppets, or crayons and paper. In addition the disclosure may be recanted at a subsequent time or to another individual.

Diagnosing sexual abuse on the basis of the physical examination is dependent on the presence of abnormal genital and/or anal findings. Changes in the genital area that are consistent with sexual abuse include tears, scars, and distortion of the hymen, with the presence of synechiae and neovascularization.[13] The presence of labial fusion obscures these findings, and necessitates the institution of treatment to allow adequate visualization of the hymenal area.

Anal findings may be absent in 50% of individuals who are either acutely or chronically sodomized. Changes of long-standing, chronic sodomy include perianal scars that are wider on the outside and taper inward, tags, changes in the appearance of the anal folds, thickening and hyperpigmentation of skin in the perianal area, loss of perianal adiposity, funneling, laxity of tone, and loss of the anal "wink."[13, 14] These changes were noted in five patients in this study.

All four children with no physical findings of sexual abuse were infants. In two there was some suspicion about molestation. One infant (Case 7) had failure to thrive, was from a chaotic household, and had been removed from the home. The second infant (Case 8) was brought in by the mother because of concern that the infant may also have been molested after an examination of the 7-year-old sister revealed findings consistent with sexual misuse. The two remaining infants had been referred for genital evaluation because of the presence of the labial fusion.

Urinary symptoms occurred in two (20%) of our patients. Others[1, 4, 15] report an incidence of urinary symptoms with labial fusion from 20% to 50%. Usual symptoms including infection, incontinence, dribbling, and tenesmus are secondary to interference with urinary outflow.[4, 11] Marked reflux of urine into the vagina with trapping of the urine above the fused labia has been demonstrated with contrast material during voiding cystourethrography.[1] Because of the high frequency of urinary symptoms, urinalysis and urine culture appear warranted in the evaluation of labial fusion.

Treatment with estrogen cream will relieve the fusion in about 90%.[5] Treatment is usually not necessary because the fusion will resolve spontaneously at puberty.[1] Jenkinson and Mackinnon[16] reported spontaneous resolution in 10 infants under 2 years of age followed up for 18 months. Early intervention is recommended if urologic symptoms exist or there is a need for adequate visualization of the hymenal area. Treatment is not without side effects, such as breast engorgement, and the labia may re-fuse with cessation of therapy. Surgical separation of the fused labia, which has been advocated by some investigators, is rarely needed, is unnecessarily traumatic, and carries a high incidence of recurrence.[5, 11]

In conclusion labial fusion is a disorder of adhesion of the labia minora. It may occur secondary to a variety of inflammatory conditions including vulvovaginitis or seborrheic or atopic dermatitis. In young infants, fecal soiling and diaper dermatitis may promote the condition. In older girls, trauma such as that associated with sexual abuse may predispose to labial fusion. The finding of labial fusion in the older girl warrants a careful anal examination and a disclosure interview by skilled personnel to determine if there is a history of sexual abuse.

REFERENCES

1. Ben-Ami T, Boichis H, Hertz M. Fused labia. Clinical and radiological findings. Pediatr Radiol 1978;7:33-5.
2. Bowles HE, Childs LS. Synechias of vulva in small children. Am J Dis Child 1943;66:258-63.
3. Glenn JF. Labial fusion and urinary infection. J Urol 1962;87:485-6.
4. Nowlin P, Adams JR, Nalle BC Jr. Vulvar fusion. J Urol 1949;62:75-9.
5. Capraro VJ, Greenberg H. Adhesions of the labia minora. Obstet Gynecol 1972;39:65-9.
6. Altchek A. Vulvovaginitis, vulvar skin disease and pelvic inflammatory disease. Pediatr Clin North Am 1981;28: 397-431.

7. Dewhurst J. Genital tract obstruction. Pediatr Clin North Am 1981;28:331-44.
8. Grumbach MM, Ducharme JR. The effects of androgens on fetal sexual development. Fertil Steril 1960;11:157-80.
9. Hammerschlag MR, Cummings M, Doraiswamy B, et al. Nonspecific vaginitis following sexual abuse in children. Pediatrics 1985;75:1028-31.
10. Vakar NR. Vulvar adhesions in young children. Pediatria 1930;14:477-82.
11. Campbell MF. Vulvar fusion. JAMA 1940;115:513-5.
12. Taylor WN. Vulvar fusion: two cases with urological aspects. J Urol 1941;45:710-4.
13. Woodling BA, Kossoris P. Sexual misuse, rape, molestation and incest. Pediatr Clin North Am 1981;28:481-99.
14. Paul DM. The medical examination in sexual offenses against children. Med Sci Law 1977;17:251-8.
15. Christensen EH, Oster J. Adhesions of labia minora (synechia vulvae) in childhood. Acta Paediatr Scand 1971; 60:709-15.
16. Jenkinson SD, Mackinnon AE. Spontaneous separation of fused labia minora in prepubertal girls. Br Med J 1984; 289:160-1.

Postmenopausal smoking, estrogen replacement therapy, and the risk of endometrial cancer

Adele L. Franks, M.D., Juliette S. Kendrick, M.D., Carl W. Tyler, Jr., M.D., and The Cancer and Steroid Hormone Study Group*

Atlanta, Georgia

Previous studies of the association between cigarette smoking and endometrial cancer have yielded inconsistent results. There is some evidence that this discrepancy may be explained by differences in menopausal status between the groups of women studied. We addressed the issue of the effects of postmenopausal smoking on endometrial cancer risk using data from the Cancer and Steroid Hormone Study, a multicenter, population-based, case-control study of gynecologic cancers in the United States. We found that smoking after natural menopause is associated with a 70% reduced risk of endometrial cancer among estrogen users and a 50% reduced risk among nonusers of estrogen. These findings are consistent with previously proposed biologic effects of smoking on estrogen metabolism, which may have important clinical implications. (Am J Obstet Gynecol 1987;156:20-3.)

Key words: Endometrial cancer, estrogen replacement therapy, smoking, estrogen metabolism

It is now widely agreed that endometrial cancer is associated with estrogen stimulation of the endometrium.[1-3] Several studies have shown that women who smoke have earlier menopause,[4-6] an increased risk of osteoporosis,[7-9] and decreased urinary estrogens,[10] suggesting that smokers have reduced estrogen. Thus, since there is no evidence for a direct carcinogenic effect of cigarette smoking on the endometrium, smokers might be expected to have a reduced risk of endometrial cancer by virtue of diminished estrogen stimulation.

From the Division of Reproductive Health, Center for Health Promotion and Education, Centers for Disease Control.
Supported by interagency agreement 3-YO1-HD-8-1037 between the Centers for Disease Control and the National Institute of Child Health and Human Development, with additional support from the National Cancer Institute.
Received for publication January 29, 1986; accepted August 12, 1986.
Reprints are not available.
**Individual members of the study group are listed at the end of the article.*

Previous studies of this relationship have yielded inconsistent results, and only three studies have controlled for important potential confounding factors.[11-13] Weiss et al.[11] found a significant negative association between "ever smoking" history and endometrial cancer (relative risk = 0.4, 95% confidence interval = 0.2 to 0.7), whereas Tyler et al.[12] found a slightly lowered risk which was not statistically significant (relative risk = 0.8, 95% confidence interval = 0.7 to 1.1). Both studies showed that cigarette smoking reduced the elevated risk of endometrial cancer among women who used estrogens. Since Weiss et al. studied women 50 to 74 years old and Tyler et al. studied women 20 to 55 years old, the difference in smoking effect may be related to factors that are age dependent, in particular, to menopausal status.

A recent study by Lesko et al.[13] has suggested that menopausal status may be an important modifier of the effects of smoking. They found a significantly reduced risk of endometrial cancer (relative risk = 0.5, 95%

confidence interval = 0.3 to 0.8) among women who were currently smoking at least 25 cigarettes per day. When premenopausal and postmenopausal women were considered separately, the protective effect of smoking was confined to the postmenopausal women. The effect of smoking in relation to exogenous estrogen use was not reported.

To address the hypothesis that both postmenopausal smoking and postmenopausal estrogen use affect endometrial cancer risk, we analyzed data from a subset of women enrolled in the Cancer and Steroid Hormone Study. The previous analysis[12] used the entire data set to address the question of cumulative lifetime effects of cigarette smoking and did not consider the temporal relation of smoking to menopause.

Material and methods

The details of this study have been described elsewhere.[12, 14] Cases were women who were 20 to 55 years old with histologically confirmed epithelial endometrial cancer newly diagnosed between December 1, 1980, and December 31, 1982, in six geographic areas (two of the original eight centers did not participate in the histologic review). Controls were women of the same age range identified by calling randomly selected telephone numbers from the same geographic areas as the cases. For this analysis we selected only postmenopausal women over the age of 40 (106 case subjects, 528 controls). These were women who had undergone a natural menopause, and whose last menstrual period occurred after the age of 40 and at least 6 months before diagnosis or interview. (This excluded perimenopausal women, who were included in the postmenopausal category in the previous analysis).

Postmenopausal smoking was defined as smoking cigarettes after the last menstrual period, regardless of when smoking began. Women who stopped smoking before menopause were excluded (17 cases, 87 controls). Postmenopausal estrogen replacement therapy was defined as first use of noncontraceptive estrogen beginning after age 40 and continuing after the last menstrual period. Women whose only use of noncontraceptive estrogen occurred before menopause were excluded (10 cases, 25 controls). This left 495 women (79 cases, 416 controls) for analysis. Nonsmokers were women who reported having smoked less than a total of 100 cigarettes in their lifetime, and nonusers of estrogen replacement therapy had never used noncontraceptive estrogens for 3 consecutive months or more.

Logistic regression was used to derive odds ratios (as relative risk estimates) and confidence intervals[15] for the exposures of interest. Relative risk estimates were adjusted both individually and simultaneously for the following factors considered a priori to potentially confound the results: age and age at menopause (as continuous variables), race, Quetelet's adiposity index (weight/height squared), hypertension, diabetes, infertility, parity, history of oral contraceptive use, and geographic region (as categorical variables). None of these adjustments appreciably altered the risk estimates. Therefore the unadjusted relative risk estimates are presented, with test-based confidence intervals.[16]

To evaluate the possible modification of smoking effects by previous oral contraceptive use or obesity, data were stratified according to these factors.

Table I. Selected characteristics of postmenopausal endometrial cancer cases and controls

Characteristic	*Cases (%) (n = 79)*	*Controls (%) (n = 416)*
Age (yr)		
40-49	11	17
≥50	89	83
Race		
White	95	85
Black/other	5	15
Parity		
0	30	11
≥1	70	89
Quetelet's index*		
<2.50	51	77
≥2.50	48	23
Unknown	1	0
Age at menopause (yr)		
<50	70	69
≥50	30	31
History of		
Hypertension	42	21
Oral contraceptive use	28	38
Diabetes	6	2

*Weight (kg)/[height (cm)]^2.

Results

Cases were more likely than controls to be older, white, nulliparous, obese, and hypertensive and to have never used oral contraceptives (Table I). Smoking after menopause was associated with a significantly reduced risk of developing endometrial cancer, with smokers having half the risk of nonsmokers (Table II). When stratified by use of estrogen replacement therapy (Table III), a similar smoking effect was present both among nonusers and users of estrogen replacement therapy (reference group 1 column). Smokers who did not use estrogen replacement therapy had a 50% reduction in endometrial cancer risk compared with nonsmokers, whereas smokers who did use estrogen replacement therapy had a 70% reduction in risk.

The separate effects of single or joint exposures to postmenopausal smoking and estrogen replacement therapy, compared with exposure to neither, can be seen in the reference group 2 column in Table III.

Table II. Epithelial endometrial cancer risk associated with postmenopausal smoking

Smoking status	*Cases*	*Controls*	*Relative risk*	*95% Confidence interval*
Nonsmokers	50	188	1.0	Referent
Postmenopausal smokers	29	228	0.5	0.3-0.8

Table III. Epithelial endometrial cancer risk associated with postmenopausal smoking, stratified by postmenopausal estrogen replacement therapy

Estrogen replacement therapy and smoking status	*Cases*	*Controls*	*Reference group 1**		*Reference group 2†*	
			Relative risk	*95% Confidence interval*	*Relative risk*	*95% Confidence interval*
Nonusers of estrogen replacement therapy						
Nonsmokers	37	172	1.0	Referent	1.0	Referent
Postmenopausal smokers	23	200	0.5	0.3-0.9	0.5	0.3-0.9
Postmenopausal estrogen replacement users						
Nonsmokers	13	16	1.0	Referent	3.8	1.7-8.2
Postmenopausal smokers	6	28	0.3	0.1-0.8	1.0	0.4-2.6

*Based on nonsmokers in each estrogen replacement therapy category as reference group.

†Based on nonsmokers who were nonusers of estrogen replacement therapy as reference group for all comparisons.

Women who used estrogen replacement therapy but did not smoke had a significantly elevated risk (relative risk = 3.8) of endometrial cancer. Women who used estrogen replacement therapy and smoked had the same risk as the nonusers who did not smoke (relative risk = 1.0).

We were unable to evaluate the risk of endometrial cancer according to daily dose of cigarette smoking after menopause, because the only dose information acquired was the lifetime daily average number of cigarettes smoked. There was no consistent trend in endometrial cancer risk according to lifetime average amount smoked each day.

Stratification by obesity and previous oral contraceptive use revealed no appreciable stratum-specific differences in the effects reported.

Comment

Our results show that smoking cigarettes after natural menopause is associated with a decreased risk of developing endometrial cancer for postmenopausal women 40 to 55 years old. The increased risk of endometrial cancer associated with postmenopausal estrogen replacement therapy is reduced in postmenopausal smokers in such a way that women with both exposures have the same risk as women with neither.

Despite the relatively small number of postmenopausal women in our study, our findings are consistent with previous research.[11, 13] We do not believe confounding accounts for our findings, since adjustment for known endometrial cancer risk factors did not alter our results.

The protective effect of smoking emerged more clearly in a subset of women who smoked after natural menopause than in the larger group of women with unspecified exposure intervals.[12] Comparison of currently smoking premenopausal women (22 cases, 474 controls) with nonsmoking premenopausal women (50 cases, 753 controls) yielded a smaller reduction in risk which was not statistically significant (relative risk = 0.7, 95% confidence interval = 0.4 to 1.2). Because premenopausal women are a hormonally heterogeneous group, their risk of endometrial cancer may not be uniformly affected by smoking.

Baron[17] has suggested that the antiestrogenic effects of smoking in women may be mediated by induction of hepatic microsomal enzymes similar to those induced by smoking in laboratory animals.[18] Our data are consistent with an antiestrogenic smoking effect among postmenopausal users of exogenous estrogen replacement therapy. This supports the possibility of a peripheral, and extraovarian, biologic mechanism such as induction of estrogen metabolizing enzymes. Such a peripheral mechanism may be operative only while the inducing exposure is present. Thus the antiestrogenic effect of cigarette smoking may be reversible after cessation of smoking. In our data, postmenopausal women who quit smoking before menopause (and thus were excluded from the data shown) had a diminished endometrial cancer risk intermediate between, but not significantly different from, the nonsmokers and postmenopausal smokers (compared with nonsmokers relative risk = 0.7 and 95% confidence interval = 0.4 to 1.3).

Even with evidence that cigarette smoking is associated with a significantly reduced risk of endometrial

cancer among postmenopausal women, women should not smoke for this or any other reason, in view of the serious morbidity and mortality of common smoking-attributable diseases.

We believe, however, that such findings have potentially important clinical implications. There is mounting evidence to suggest that use of estrogen replacement therapy by postmenopausal women decreases their risk of ischemic heart disease[19, 20] and osteoporosis[8, 9, 21-23] and that these protective effects are at least in part dose dependent. The finding of this analysis and others,[11, 13] that postmenopausal smoking is associated with decreased estrogen effects on the endometrium, is consistent with smoking-induced enhancement of estrogen metabolism. By such a mechanism, smoking may also diminish the effects of estrogen on other end organs such as coronary arteries and bone.[8] If so, postmenopausal women who cannot be persuaded to stop smoking may need higher doses of estrogen than nonsmokers to achieve comparable therapeutic effects.

Individual members of The Cancer and Steroid Hormone Study Group are as follows: Principal Investigator: George L. Rubin, M.B. Project Director: Phyllis A. Wingo, M.S. Project Associates: Nancy C. Lee, M.D., Michele G. Mandel, B.A., and Herbert B. Peterson, M.D. Data Collection Centers Principal Investigators: Atlanta—Raymond Greenberg, M.D.; Connecticut—J. Wister Meigs, M.D., and W. Douglas Thompson, Ph.D.; Detroit, Michigan—G. Marie Swanson, Ph.D.; Iowa—Elaine Smith, Ph.D.; New Mexico—Charles Key, M.D., and Dorothy Pathak, Ph.D.; San Francisco—Donald Austin, M.D.; Seattle—David Thomas, M.D.; Utah—Joseph Lyon, M.D., and Dee West, Ph.D. Pathology Review Principal Investigators: Fred Gorstein, M.D., Robert McDivitt, M.D., and Stanley J. Robboy, M.D. Project Consultants: Lonnie Burnett, M.D., Robert Hoover, M.D., Peter M. Layde, M.D., M.Sc., Howard W. Ory, M.D., M.Sc., James J. Schlesselman, Ph.D., David Schottenfeld, M.D., Bruce Stadel, M.D., Linda A. Webster, M.S.P.H., and Colin White, M.B., B.S. Pathology Consultants: Walter Bauer, M.D., William Christopherson, M.D., Deborah Gersell, M.D., Robert Kurman, M.D., Allen Paris, M.D., and Frank Vellios, M.D.

REFERENCES

1. Weiss NS, Szekely DR, English DR, Schweid AI. Endometrial cancer in relation to patterns of menopausal estrogen use. JAMA 1979;242:261-4.
2. Jick H, Watkins RN, Hunter JR, et al. Replacement estrogens and endometrial cancer. N Engl J Med 1979; 300:218-22.
3. Kelsey JL, LiVolsi VA, Holford TR, et al. A case-control study of cancer of the endometrium. Am J Epidemiol 1982;116:333-42.
4. Jick H, Porter J, Morrison AS. Relation between smoking and age of natural menopause. Lancet 1977;1:1354-5.
5. Kaufman DW, Slone D, Rosenberg L, Miettinen OS, Shapiro S. Cigarette smoking and age at natural menopause. Am J Public Health 1980;70:420-2.
6. Willett W, Stampfer MJ, Bain C, et al. Cigarette smoking, relative weight, and menopause. Am J Epidemiol 1983; 117:651-8.
7. Daniell HW. Osteoporosis of the slender smoker. Arch Intern Med 1976;136:298-304.
8. Williams AR, Weiss NS, Ure CL, Ballard J, Daling JR. Effect of weight, smoking and estrogen use on the risk of hip and forearm fractures in postmenopausal women. Obstet Gynecol 1982;60:694-9.
9. Paganini-Hill A, Ross RK, Gerkins VR, Henderson BE, Arthur M, Mack TM. Menopausal estrogen therapy and hip fractures. Ann Intern Med 1981;95:28-31.
10. MacMahon B, Trichopoulos D, Cole P, Brown J. Cigarette smoking and urinary estrogens. N Engl J Med 1982; 307:1062-5.
11. Weiss NS, Farewell VT, Szekely DR, English DR, Kiviat N. Oestrogens and endometrial cancer: effect of other risk factors on the association. Maturitas 1980;2:185-90.
12. Tyler CW, Webster LA, Ory HW, Rubin GL. Endometrial cancer: how does cigarette smoking influence the risk of women under age 55 years having this tumor? AM J OBSTET GYNECOL 1985;151:899-905.
13. Lesko SM, Rosenberg L, Kaufman DW, et al. Cigarette smoking and the risk of endometrial cancer. N Engl J Med 1985;313:593-6.
14. The Centers for Disease Control Cancer and Steroid Hormone Study. Long-term oral contraceptive use and the risk of breast cancer. JAMA 1983;249:1591-5.
15. Schlesselman JJ. Case-control studies: design, conduct, analysis. New York: Oxford University Press, 1982; 235-57.
16. Rothman KJ, Boice JD. Epidemiologic analysis with a programmable calculator. Bethesda, National Institutes of Health publication No. 79-1649, 1979, p 6.
17. Baron JA. Smoking and estrogen-related disease. Am J Epidemiol 1984;119:9-22.
18. Conney AH. Pharmacological implications of microsomal enzyme induction. Pharmacol Rev 1967;19:317-366.
19. Ross RK, Paganini-Hill A, Mack TM, Arthur M, Henderson BE. Menopausal oestrogen therapy and protection from death from ischemic heart disease. Lancet 1981;1: 858-60.
20. Paganini-Hill A, Ross RK, Henderson BE. Protection from acute myocardial infarction among users of estrogen replacement therapy. Paper presented at the 18th Annual Meeting of the Society for Epidemiologic Research, Chapel Hill, NC, June 1985.
21. Weiss NS, Ure CL, Ballard JH, Williams AR, Daling JR. Decreased risk of fractures of the hip and lower forearm with postmenopausal use of estrogen. N Engl J Med 1980;303:1195-8.
22. Horsman A, Jones M, Francis R, Nordin C. The effect of estrogen dose on postmenopausal bone loss. N Eng J Med 1983;309:1405-7.
23. Lindsay R, Hart DM, Clark DM. The minimum effective dose of estrogen for prevention of postmenopausal bone loss. Obstet Gynecol 1984;63:759-63.

The efficacy of intrapartum electronic fetal monitoring

Stephen B. Thacker, M.D.
Atlanta, Georgia

With basic methodologic criteria as a framework, this report assesses the quality of the seven randomized controlled clinical trials conducted in five countries to compare a policy of routine electronic fetal monitoring with a policy of fetal heart rate monitoring by auscultation. One trial found a statistically significant decrease in the occurrence of neonatal seizures in the electronic fetal monitoring group. The trials demonstrated no other statistically significant benefit associated with the use of electronic fetal monitoring, but most reported significant increases in the rates of abdominal and vaginal operative deliveries associated with electronic fetal monitoring. Taken together, the seven trials provide valuable information about the routine use of intrapartum electronic fetal monitoring; they do not demonstrate that it is a useful screening procedure for all women in labor. (Am J Obstet Gynecol 1987;156:24-30.)

Key words: Electronic fetal monitoring, randomized controlled trials

A 1979 literature review on the efficacy and safety of electronic fetal monitoring coauthored by Banta and Thacker[1] concluded that there was insufficient evidence at that time to recommend the routine use of electronic fetal monitoring. In addition, we concluded that there was low predictive value positive for electronic fetal monitoring, a high cost to the individual and society, and a premature diffusion of this technology into routine clinical practice. Four randomized controlled clinical trials completed at that time failed to demonstrate a decrease in morbidity and mortality associated with the use of electronic fetal monitoring but did show a twofold increase in the rate of cesarean delivery among patients monitored electronically.[2-5] Because the available evidence suggested a possible benefit for the use of electronic fetal monitoring in selected high-risk pregnancies, we recommended that randomized controlled clinical trials be undertaken to test that hypothesis.

The randomized controlled clinical trial is generally accepted as the ideal method to assess the efficacy of clinical interventions in human populations.[6] As with any human endeavor, however, trials are subject to flaws in conduct and errors in implementation.[7] This report systematically assesses the seven randomized controlled clinical trials conducted to date to evaluate the routine use of electronic fetal monitoring[2-5, 8, 9] (Neldam S, Osler M, Hansen P, et al., unpublished observations).

From the Division of Surveillance and Epidemiologic Services, Epidemiology Program Office, Centers for Disease Control.
Received for publication February 18, 1986; revised July 25, 1986; accepted August 14, 1986.
Reprint requests: Stephen B. Thacker, M.D., Center for Environmental Health, Centers for Disease Control, Atlanta, GA 30333.

Assessing a clinical trial

Understanding of the 12 criteria given in Table I is essential to adequate interpretation of the conduct and analysis of an randomized controlled clinical trial.[10, 11] In the completion of Table I, an effort was made to obtain previously unpublished information from the authors to give a more complete picture of the available knowledge.

Prior hypotheses and statistical power. Greatest emphasis should be given to results related to those hypotheses for which a study is designed. Prior hypotheses were precisely stated only in the Dublin trial, although general goals were implicit in the other six. Statistical power (the probability that a study will detect a true difference between two populations) was calculated and sample size was estimated only in the Dublin study.

Eligibility criteria. Eligibility criteria for entry into the study were explicit in all but the first Melbourne study, for which no definition of "high risk" was given.

Allocation of subjects. Randomization both eliminates selection bias and enables the proper use of statistical methods.[12] The investigators in all trials allocated women into electronic fetal monitoring and auscultation groups by means of formal randomization with opaque, sealed envelopes except in Melbourne (see below) and in Copenhagen, where the weeks of participation were randomly allocated. The weekly allocation procedure used by the Danish investigators is subject to selection bias (for example, the decision to induce labor could be affected by the physician's or midwife's desire to assign a patient to an experimental [or control] group).

Because randomization allocates subjects in an unbiased manner, potentially confounding variables tend

Table I. Assessment of reports of seven clinical trials evaluating the efficacy of routine electronic fetal monitoring during labor

	Denver 1976	*Melbourne 1976*	*Sheffield 1978*	*Denver 1979*	*Melbourne 1981*	*Copenhagen 1981*	*Dublin 1985*
Prior hypotheses	N	N	N	N	N	N	Y
Power calculation	N	N	N	N	N	N	Y
Eligibility criteria	Y	N	Y	Y	Y	Y	Y
Allocation	RN	RN	RN	RN	*	RW	RN
Withdrawals	Y	N	N	Y	N	Y	U
Description of regimen	Y	Y	Y	Y	Y	Y	Y
Adherence to regimen							
Doctor/midwives	U	N	U	U	U	Y	Y
Blinding							
Pediatricians	Y	Y	N	N	N	N	Y,N
Statisticians	N	N	N	N	N	Y	Y
Unambiguous end points	S	S	S	S	S	S	S
Appropriate statistics							
Main effects	Y	Y	Y	Y	Y	Y	Y
Subgroups	N	N	N	N	N	N	Y
Complications of monitoring	Y	Y	Y	Y	Y	Y	Y

Y, Yes; N, no; U, unclear; S, some; RN, random numbers, sealed envelopes; RW, random weeks, sealed envelopes.
*Randomization was attempted but failed in the major study hospital.

to be equally distributed in the experimental and control groups. Nevertheless, it is still useful to assess whether known risk factors and confounders were distributed equally in the study and control groups. This postrandomization assessment was attempted with varying rigor in each of the trials.

Withdrawals. Withdrawals were clearly dealt with only in the Copenhagen and Dublin studies. There were apparently no withdrawals in the Sheffield study, but recognition of the importance of this fact was not apparent in that report. Of the 13,025 women who were eligible for the Dublin trial, 61 (0.5%) were inadvertently not randomized but on review of their hospital records were not found to differ from those included in the study. In the Danish study, 349 women (31%) refused to participate because of a preferred method of intrapartum surveillance (238 of these women had delivery conducted with auscultation, 111 with electronic fetal monitoring).

Retaining the groups as they were originally allocated is essential for the primary analyses, although additional analyses with clearly specified exclusions can be used for supplementary information.[11] Excluding withdrawals, even those beyond the control of the investigators, can vitiate the bias-eliminating effects of randomization.[10] Any other analyses must be considered as supplementary or as hypothesis-generating exercises. Such analyses are often useful but should not be used for hypothesis testing.

Study regimens. Study regimens for both experimental and control groups were well described in each trial. Postrandomization patient compliance was uniformly high. Adherence to the study regimen is crucial but often difficult to assess, unless the investigators make careful efforts either to document deviations from the protocol or to design methods to quantify such deviations. Only the Dublin investigators made extensive efforts to assess adherence to the study regimen, although in both Denver trials as well as the trial in Sheffield the doctors and midwives apparently adhered closely to the regimen. The regimen was seriously breached in both Australian trials. In the first trial one of the eight study physicians withdrew his patients, introducing selection bias. Also, information bias was introduced, since scalp blood measurements were performed only between 9:00 AM and 5:00 PM in that trial. In the second Melbourne trial the cards for allocation were not in sealed envelopes, and the randomization was apparently lost. This led to an increased proportion of primigravid women in the electronic fetal monitoring group, and efforts by the investigators to adjust for this and other unrecognized biases were not successful.

The appropriate use of a technology is a crucial aspect of any study. Although explicit decision pathways based on electronic fetal monitoring results can be constructed, the type and timing of intervention are based ultimately on clinical judgment. This reflects the real world, and one must recognize that these randomized controlled clinical trials measure the effectiveness of management practice (pragmatic trials) rather than the potential efficacy of a procedure in an ideal setting.[12]

"Blinding" of evaluation by pediatricians and statisticians. The conduct of randomized controlled clinical trials is subject to bias when those assessing outcomes are aware of the assignment of a patient to the experimental or control group. Although it was not practical

Table II. Results of seven clinical trials assessing the efficacy of routine electronic fetal monitoring during labor

Characteristics	*Denver 1976*		*Melbourne 1976*		*Sheffield 1978*		*Denver 1979*			*Melbourne 1981*	
	E-I	*A*	*E-II*	*A*	*E-I*	*A*	*E-I*	*E-II*	*A*	*E-II*	*A*
No. of subjects											
Pregnant women	242	241	175	175	253	251	233	230	232	445	482
Infants	242	241	175	175	253	251	233	230	232	445	482
Maternal risk status	High		High		Low			High		Low	
Fetal scalp blood sampling	No	–	Yes	–	No	–	No	Yes	–	Yes	–
Cesarean deliveries											
Total No.	40	16	39	24	24	11	41	26	13	18	10
%	16.5	6.6	22.3	13.7	9.5	4.4	17.6	11.3	5.6	5.8	2.7
No. with fetal distress	18	3	16	9	4	3	16	8	1	NA	NA
%	7.4	1.2	9.1	5.1	1.6	1.2	6.9	3.5	0.4	NA	NA
Operative vaginal deliveries											
No.	60	78	70	67	71	78	64	54	54	120	101
%	24.8	32.4	40.0	38.3	28.1	31.1	27.5	23.5	23.3	27.0	20.7
1 min Apgar score <7											
No.	31	29	28	25	24	24	41	35	33	39	40
%	12.8	12.0	16.0	14.3	9.6	9.5	17.5	15.2	14.2	8.3	8.8
1 min Apgar score <4											
No.	NA	NA	4	6	4	7	14	10	11	4	6
%	NA	NA	2.3	3.4	1.6	2.8	6.0	4.3	4.7	0.9	1.2
Neonatal seizures											
No.	2	2	0	4	0	1	2	0	2	0	0
Rate/1000	8.3	8.3	0.0	22.9	0.0	4.0	8.6	0.0	8.6	0.0	0.0
No. receiving oxytocin	NA	NA	0	NA	0	NA	NA	0	NA	0	0
Neonatal intensive care											
No. of admissions	35	28	11	30	45	43	23	29	29	59	48
%	14.5	11.6	6.3	17.1	17.8	17.1	9.9	12.6	12.5	13.3	10.0
Mean length of stay (days)	NA	NA	3.1§	3.8§			NA	NA	NA	NA	NA
Perinatal death											
Rate/1000	8.3	4.1	5.7	5.7	0.0	4.0	8.6	4.3	0.0	2.2	0.0
Stillbirths	0	0	0	1	0	0	0	0	0	0	0
Neonatal deaths	2	1	1	0	0	1	2	1	0	1	0

E-I = Group with electronic fetal monitoring only (experimental); E-II = group with electronic fetal monitoring and scalp blood sampling (experimental); A = auscultation group (control); NA = data not available.

*Excluded women with diabetes mellitus Classes B, C, D, and F.

†Excluded women with abnormal amniotic fluid on admission.

‡Data collected on first 10,094 pregnancies; complete on 10,035.

§Data could not be verified by author.

in these studies to "blind" either patients* or clinicians during labor, it was feasible and desirable to "blind" the assessment of infant outcome. The Denver and Dublin investigators made careful efforts to "blind" the pediatrician who assessed the newborn infants as to allocation of subjects to experimental and control groups, but only the Dublin group prespecified statistical analysis so as to avoid bias. Statisticians were "blinded" as to allocation of newborn infants in the Copenhagen study.

Unambiguous outcomes. When complete blinding is not possible, which was the case in these randomized controlled clinical trials, bias can be decreased by using unambiguous outcomes such as death. Even in this case, however, the attribution of the cause of death could have been biased. A death secondary to fetal distress could, for example, be attributed inappropriately to a cephalopelvic disproportion. Similarly, the increase in the rate of cesarean delivery associated with electronic fetal monitoring that was consistently detected in the trials must be interpreted in light of the fact that the indications for operative delivery are variable and potentially subject to observer bias.

Statistical analysis. This remains a source of confusion in the clinical literature. Clear presentation of the simple distribution of results in both experimental and control groups is essential but is sometimes overlooked. Tests of statistical significance should be presented for major end points, with a clear description of the statistical method. Results should be presented as relative risks or risk differences with appropriate confidence intervals.[13]

Post hoc analysis of hypotheses inspired by looking at subgroup results (data dredging) should not include conventional tests of statistical significance. All the trials of routine electronic fetal monitoring used appropriate

*An attempt to "blind" the study as to allocation of the patients was made in the first Denver trial.[3]

Copenhagen 1981		*Dublin 1985*	
E-II	*A*	*E-II*	*A*
482	487	6474	6490
485	493	6530	6554
All*		All†	
Yes	–	Yes	–
28	18	158	144
5.7	3.7	2.4	2.2
8	7	25	10
1.6	1.4	0.4	0.2
85	64	528	407
17.6	13.1	8.2	6.3
28	32	216‡	227‡
5.8	6.5	4.3	4.5
5	7	62	57
1.0	1.4	1.0	0.9
0	0	12	27
0.0	0.0	1.8	4.1
0	0	4	19
51	49	547	543
10.5	9.9	8.4	8.3
4.0	5.7	NA	NA
0.0	2.1	2.1	2.1
0	1	3	2
0	0	11	12

statistics to assess main effects (although only in the Dublin trial were these main effects delineated). Only the Dublin authors calculated confidence intervals and demonstrated an understanding of the distinction between testing of prior hypotheses and the generation of new hypotheses by post hoc analysis.

Complications. While none of the trials were designed explicity to assess the complications of routine electronic fetal monitoring, all investigators measured some of the potentially adverse effects of the procedure, in particular increased rates of cesarean delivery. Only the Dublin trial was large enough, however, to detect differences in rates of less common maternal and neonatal complications previously attributed to electronic fetal monitoring. These investigators detected a statistically significant increase in the occurrence of genital tract infections among women in the electronic fetal monitoring group.

Assessing reports of clinical trials

If one were limited to published data, less could be said about the quality and comparability of the seven trials. To demonstrate this point the published reports of the trials were assessed quantitatively, with use of a previously standardized method for evaluating trials.[11] The method was modified slightly for the assessment of electronic fetal monitoring. A score of >80% is considered good, and occasionally a report is rated >90%. The Dublin trial scored highest for overall quality (86%). Three other trials scored >50%: Sheffield (57%), the second Denver trial (54%), and the Copenhagen study (51%). The other studies scored lower: the first Denver trial scored 45%; the second Melbourne trial, 32%; and the first Melbourne trial, 29%. With addition of unpublished information, each trial might have scored higher. This evaluation emphasizes the importance of adequate reporting of both methods and results. In this instance the published literature leaves the reader with concerns about the quality of all of the initial six trials, especially the first Melbourne trial, which first suggested that intrapartum electronic fetal monitoring may decrease the occurrence of adverse neurological sequelae.

Analysis of the results of the seven trials assessing intrapartum electronic fetal monitoring

None of the initial six trials demonstrated a statistically significant decrease in the rate of perinatal death, stillbirth, neonatal death, 1-minute Apgar score of <7, 1-minute Apgar score of <4, or neonatal intensive care admissions with the use of electronic fetal monitoring (Table II). Only the first Melbourne trial showed a large (although not statistically significant) decrease in neonatal seizures associated with electronic fetal monitoring, as well as a possible decrease in the rate of admission for neonatal intensive care. All of the initial six trials showed an increased rate of cesarean delivery in the electronic fetal monitoring group. The results regarding total operative deliveries were mixed, with increases in the rates of operative deliveries (that is, abdominal and forceps deliveries) in the electronic fetal monitoring group in both Melbourne trials, the second Denver trial, and the Copenhagen study, but decreases associated with electronic fetal monitoring use in the Denver and Sheffield trials.

Pooled analyses. To use trial data more efficiently, results from similar studies can be pooled for analysis.[14] This is particularly useful when trials are too small to detect important effects of an intervention, although one must interpret the results of pooling with caution.[15] In addition to clinical and epidemiologic considerations, pooling is not appropriate in circumstances in which results vary widely. Heterogeneity tests quantitatively measure the extent of outcome variability in trials.[14] In this report we pool the results of the 3928 women in the first six studies.

There was no statistical reason for not pooling data from the six initial studies on Apgar score, neonatal intensive care unit admission, perinatal death, neonatal

Table IIIA. Results of six controlled trials assessing the efficacy of routine electronic fetal monitoring in labor

Trial	*Apgar score <7 at 1 min*			*Apgar score <4 at 1 min*			*Neonatal seizures*		
	E	*A*	*V*	*E*	*A*	*V*	*E*	*A*	*V*
Denver 1976	31	29	13.16	NA	NA	–	2	2	0.99
Melbourne 1976	28	25	11.24	4	6	2.44	0	4	0.99
Sheffield 1978	24	24	10.88	4	7	2.70	0	1	0.25
Denver 1979*	41	33	15.59	14	11	5.93	2	2	0.99
Denver 1979*	35	33	14.53	10	11	5.02	0	2	0.50
Melbourne 1981	39	40	18.06	4	6	2.49	0	0	0.00
Copenhagen 1981	28	32	14.09	5	7	2.97	0	0	0.00

NICU = Neonatal intensive care unit; E = electronic fetal monitoring group (experimental); A = auscultation group (control); V = variance; NA = data not available.

*Two experimental groups, with and without fetal scalp blood sampling, were compared to an auscultation group in the second Denver trial.

Table IIIB. Pooled data from the six controlled trials

	Pooled relative risk	*95% Confidence interval*	*Test for hetrogeneity*
Apgar score <7	1.07	0.88-1.30	$x_6^2 = 1.28$ (NS)
Apgar score <4	0.87	0.56-1.30	$x_5^2 = 1.84$ (NS)
Neonatal seizures	0.39	0.14-1.08	$x_6^2 = 3.75$ (NS)
NICU admissions	1.01	0.84-1.22	$x_6^2 = 12.15$ (NS)
Perinatal deaths	1.73	0.53-5.64	$x_6^2 = 5.51$ (NS)

Table IVA. Results of six controlled trials assessing the impact of routine electronic fetal monitoring on the conduct of labor

Trial	*Cesarean deliveries*			*Operative vaginal deliveries*			*Total operative deliveries*		
	E	*A*	*V*	*E*	*A*	*V*	*E*	*A*	*V*
Denver 1976	40	16	12.40	60	78	24.69	100	94	29.08
Melbourne 1976	39	24	12.95	70	67	20.90	109	91	21.49
Sheffield 1978	24	11	8.16	71	78	26.29	95	89	29.26
Denver 1979*	41	13	11.96	64	54	22.06	105	67	27.15
Denver 1979*	26	13	8.95	54	54	20.73	80	67	25.11
Melbourne 1981	18	10	6.79	120	101	42.06	138	111	45.51
Copenhagen 1981	28	18	10.97	85	64	31.55	113	82	38.98

E = Electronic fetal monitoring (experimental); A = auscultation (control); V = variance.

*Two experimental groups, with and without fetal scalp blood sampling, were compared to an auscultation group in the second Denver trial.

Table IVB. Pooled data from the six controlled trials

	Pooled relative risk	*95% Confidence interval*	*Test for heterogeneity*
Cesarean deliveries	2.17	1.72-2.73	$x_6^2 = 4.05$ (NS)
Operative vaginal deliveries	1.10	0.95-1.27	$x_6^2 = 11.81$ (NS)
Total operative deliveries	1.42	1.24-1.62	$x_6^2 = 7.40$ (NS)

seizures, and operative deliveries (Tables III and IV). Slight but nonsignificant increases in the electronic fetal monitoring group were found in the number of Apgar scores of <7 and in the perinatal death rate. There were nonsignificant increases in the auscultation group in the number of Apgar scores of <4 and in the number of neonatal seizures. There were significant increases in the electronic fetal monitoring group in the rate of cesarean delivery but a nonsignificant increase in the total rate of operative delivery—both vaginal and abdominal.

Looking only at those randomized controlled clinical trials involving high-risk pregnancies (the Denver trials and the first Melbourne trial), one finds a nonsignificant increase in the electronic fetal monitoring group in the rate of perinatal death (pooled relative risk [RR] =

NICU admissions			*Perinatal deaths*		
E	*A*	*V*	*E*	*A*	*V*
35	28	13.72	2	1	0.75
40	29	9.08	1	1	0.50
45	43	18.19	0	1	0.25
23	29	11.57	2	0	0.50
29	29	12.71	1	0	0.25
59	48	23.65	1	0	0.12
51	49	22.44	0	1	0.25

2.72; 95% confidence interval [CI] = 0.68, 10.88) and infants with Apgar scores of <7 (pooled RR = 1.15; 95% CI = 0.88, 1.50) and nonsignficant decreases in the rates of neonatal intensive care unit admissions, (pooled RR = 0.84; 95% CI = 0.63, 1.12) and neonatal seizures (pooled RR = 0.42; 95% CI = 0.15, 1.20). There were statistically significant increases in the electronic fetal monitoring group in the rate of cesarean deliveries (RR = 2.48; 95% CI = 1.78, 3.16) but essentially no difference in the rate in total operative deliveries in association with high-risk pregnancies (pooled RR = 0.97, 95% CI = 0.79, 1.19).

Finally, in pooling data from the trials in which fetal scalp blood sampling was used to complement electronic monitoring, there were lower but still statistically significant increases in the cesarean delivery rate (pooled RR = 1.81, 95% CI = 1.33, 2.47) and total operative deliveries (pooled RR = 1.47, 95% CI = 1.24, 1.74) in the electronic fetal monitoring group.

The Dublin trial. The Dublin trial of nearly 13,000 subjects demonstrated a decrease in the number of neonatal seizures in the electronic fetal monitoring group. When the data were stratified, this difference in the rate of neonatal seizures in the experimental and control groups was associated with the use of oxytocin in prolonged labor. Six of the 39 infants with seizures—three in each group—showed neurological sequelae at 1 year of age. There were no differences in the rates of perinatal death, stillbirth, or neonatal death. There was no statistically significant difference in the rate of low Apgar score, in the rate of admission to neonatal intensive care, or in other types of neonatal morbidity. Although the increase in cesarean delivery associated with electronic fetal monitoring was not statistically significant, the rate of total operative deliveries was significantly increased in the electronic fetal monitoring group.

Comment

Two important issues are addressed in this report. First, there are specific conclusions that one can draw from the evidence in these seven trials relevant to both clinical practice and further research. Second, while the randomized controlled clinical trial is the most powerful tool available to assess a clinical practice such as electronic fetal monitoring, even this tool has limitations.

The Dublin trial demonstrates excellent design and implementation and illustrates the importance of reviewing even relatively small studies before beginning a major trial. After examining pooled data from the first four trials, the authors of that study found a suggestion for benefit of intrapartum electronic fetal monitoring and fetal acid-base assessment in the reduction of neonatal seizures. A randomized controlled clinical trial was then designed specifically to test this hypothesis. The results of the Dublin trial indicate that the use of electronic fetal monitoring decreases the rate of neonatal seizures in comparable settings, yet the possible limitation of that benefit to selected high-risk pregnancies and the long-term implications of the apparent association of neonatal seizures with the use of intrapartum oxytocin warrant further study. Finally, the trials reviewed here were designed to assess the effectiveness of electronic fetal monitoring in the reduction of specific outcomes. Hence, even if electronic fetal monitoring might better identify an abnormal fetus, intervention during labor may not improve the outcomes of interest.

Despite the excellence of the Dublin trial, one must remain cautious. First, the finding of a decrease of neonatal seizures in the electronic fetal monitoring group is not consistent with the pooled results of the previous trials. Second, even in the Dublin trial the long-term clinical implications of seizures remain unclear. As the authors point out, results of their stratified analyses suggest that the benefits of electronic fetal monitoring are restricted to an association with protracted labor and with women given oxytocin. They state therefore that selective rather than universal use of electronic fetal monitoring may be preferable. Third, one cannot extrapolate from a single trial to other settings in which both the patient population and the clinical practices may vary. The unique circumstances of a clinical trial are likely to result in more careful maternal-fetal surveillance both in the auscultation and electronic fetal monitoring groups than may be practical in other settings. Certainly, an active policy of induction of labor for prolonged pregnancy, the routine use of amniotomy, and the low rate of cesarean delivery (2.3%) in Dublin suggest that the clinical practice there is very different from that in the United States, where the rate of cesarean delivery exceeds 20%. The low prematurity rate in that study population and the policy of exclusion of patients with meconium must also be considered when applying these results to populations in the United States.

It is desirable therefore to interpret cautiously the findings of the Dublin randomized controlled clinical trial in the context of that practice setting. The authors of the second Melbourne trial concluded that each large institution should conduct its own randomized controlled clinical trial to account for variations in clinical practice and patient populations.[8] Although this is not feasible, it would be appropriate to conduct further randomized controlled clinical trials to test the hypothesis that electronic fetal monitoring in conjunction with fetal acid-base assessment reduces the rate of neonatal seizures and their sequelae, particularly among women receiving oxytocin to induce or augment labor.

Randomized controlled clinical trials comparable in size to the Dublin study would also help address important questions of the impact of electronic fetal monitoring on other measures of perinatal morbidity and mortality. To assess the long-term impact of this finding, additional randomized controlled clinical trials will provide a larger cohort of children who had neonatal seizures. The published literature indicates that a neonatal seizure is a serious prognostic finding, yet followup of the 39 Dublin infants with seizures who survived the neonatal period shows no difference in the outcomes of the auscultation and electronic fetal monitoring groups at 1 year of age. This suggests that there may have been a more sensitive diagnostic criterion used in the Dublin trial or that the seizures with adverse prognostic implications are not those affected by early intervention during labor and delivery.

Finally, establishing policy in clinical practice requires not only the determination that a screening procedure is effective but also that the human and monetary costs are acceptable.[16] Moreover, the effectiveness, safety, and acceptability of subsequent interventions must be considered. For example, the evidence reviewed here demonstrates a significant increase in the rates of vaginal and abdominal operative deliveries associated with electronic fetal monitoring, with or without fetal scalp blood sampling. In this light, if electronic fetal monitoring is used in essentially all pregnancies, it will have large, direct financial costs which will increase dramatically if associated with an intervention (for example, cesarean delivery) that may often be unnecessary. Before a rational decision can be made about the routine use of this technology, the value of intrapartum electronic fetal monitoring screening must be assessed in light of the potential benefit of alternative obstetric practices designed to decrease perinatal morbidity and mortality.

First, I wish to acknowledge Dr. H. David Banta for his contributions to this manuscript. In addition, I thank Professors Ian D. Cooke and Mogens Osler and Drs. Adrian Grant, Albert Haverkamp, and Peter Renou for providing background and unpublished data from the clinical trials as well as Dr. Herbert L. Peterson for comments on the manuscript.

REFERENCES

1. Banta HD, Thacker SB. Assessing the costs and benefits of electronic fetal monitoring. Obstet Gynecol Surv 1979;34:627-42.
2. Renou P, Chang A, Anderson I, et al. Interpretation of the continuous fetal heart rate monitor. Obstet Gynecol 1976;126:470-6.
3. Haverkamp AD, Thompson HE, McFee JG, et al. The evaluation of continuous fetal heart rate monitoring in high-risk pregnancy. AM J OBSTET GYNECOL 1976;125: 310-7.
4. Kelso IM, Parsons RJ, Lawrence GF, et al. An assessment of continuous fetal heart rate monitoring in labor. AM J OBSTET GYNECOL 1978;131:526-32.
5. Haverkamp AD, Orleans M, Langendoerfer S, et al. A controlled trial of the differential effects of intrapartum fetal monitoring. AM J OBSTET GYNECOL 1979;134:399-412.
6. Cochrane AL. Effectiveness and efficiency. Abingdon, United Kingdom: The Nuffield Provincial Hospitals Trust, 1971.
7. Feinstein AR, Wells CK. Randomized trial versus historical controls, scientific plagues of both houses. Trans Assoc Am Physicians 1977;90:239-44.
8. Wood C, Renou P, Oates J, et al. A controlled trial of fetal heart rate monitoring in a low-risk obstetric population. AM J OBSTET GYNECOL 1981;141:527-34.
9. MacDonald D, Grant A, Sheridan-Pereira M, et al. The Dublin randomized control trial of intrapartum fetal heart rate monitoring. AM J OBSTET GYNECOL 1985;152: 524-39.
10. Mosteller F, Gilbert JP, McPeek B. Reporting standards and research strategies for control trials. Controlled Clin Trials 1980;1:37-58.
11. Chalmers TC, Smith H Jr, Blackburn B, et al. A method for assessing the quality of a randomized control trial. Controlled Clin Trials 1981;2:31-49.
12. Schwartz D, Flamant R, Lellouch J. Clinical trials. London: Academic Press, 1980.
13. Rothman KJ. A show of confidence. N Engl J Med 1978;299:1362-3.
14. Collins R, Yusuf S, Peto R. Overview of randomized trials of diuretics of pregnancy. Br Med J 1985;290:17-23.
15. Goldman L, Feinstein AR. Anticoagulants and myocardial infarction: the problems of pooling, drowning and floating. Ann Intern Med 1979;90:92-4.
16. Garcia J, Corry M, MacDonald D, Elbourne D, Grant A. Mothers' view of continuous electronic fetal heart monitoring and intermittent ausculation in a randomized controlled trial. Birth 1985;12:79-85.

A high prevalence of chlamydial cervicitis in postmenopausal women

Tetsuo Nagashima, M.D.
Yokosuka, Japan

One hundred eighty-one healthy women were examined for *Chlamydia trachomatis* of the cervix. The results revealed a high prevalence of chlamydia-positive cases concentrated in postmenopausal ages. We would like to suggest that the primary infections occurred when the subjects were younger and that the organisms have survived for decades. (AM J OBSTET GYNECOL 1987;156:31-2.)

Key words: Chlamydial cervicitis, postmenopausal women, trachomatous conjunctivitis

Many studies on *Chlamydia trachomatis* have been made to date, and it is now known that a large number of asymptomatic infections in women are prevalent worldwide.

Clinical or epidemiologic studies of chlamydial cervical infections, however, focused mainly on sexually active women and thus resulted in a higher positive rate in younger women. Some of them revealed a much higher prevalence among populations sampled by sexually transmitted disease departments, a tendency which apparently seems plausible.

Method

During the period between November 1 and December 10, 1985, a total of 181 healthy women were examined for *C. trachomatis* in the uterine cervix during regular checkups. Their age span was 16 to 65 years, with an average of 38.0 years.

Prostitutes, contacts with men having nongonococcal urethritis and/or other sexually transmitted disease(s), and women who themselves had other sexually transmitted disease(s) (genital herpes, trichomonal vaginitis, gonorrhea, etc.) were excluded from this study, with the exception of those with candida vaginitis.

The number of those excluded was 24, their average age was 25.5 years, and the prevalence of chlamydial cervicitis was 58%.

Examinations were performed with use of the MicroTrak Chlamydia Trachomatis Direct Specimen Test (Syva Company, Palo Alto, CA 94304). Specimens were collected and fixed by me.

Results

Of the 181 cases studied, 10 positive cases were identified, for a 5.5% prevalence rate, which is comparable to the prevalence rate among the general population already reported.

Table I. Prevalence of chlamydial cervicitis in relation to the age of the women examined

Age (yr)	*No. of cases examined*	Chlamydia trachomatis–*positive*	
		n	%
16-19	9	1	11
20-29	26	2	7.7
30-39	50	0	0
40-49	70	1	1.4
50-59	21	5	23.8
60-65	5	1	20
16-65	181	10	5.5

In the age group of women under 40 years, the correlation between positive rate and age corresponded to that of the Swedish population reported on by Weström et al. in 1982.[1]

Contrary to my expectation, however, the age group over 40 years showed a much higher positive rate with seven positive cases, of which six were postmenopausal.

The results are shown in the Table I and Fig. 1.

Each case is described as follows (all women were asymptomatic, and all were Japanese): (1) A 44-year-old premenopausal woman, had a history of corneoconjunctivitis, which was diagnosed 11 years earlier and required considerable time for complete resolution. Her two children were also diagnosed at the same time as having a similar condition. (2) A 50-year-old postmenopausal woman had a history of epidemic conjunctivitis, which was diagnosed 10 years ago and required considerable time for complete resolution. One of her sons was also diagnosed at the same time as having a similar condition. (3) A 57-year-old postmenopausal woman had an uncertain history of trachomatous conjunctivitis at the age of nine or ten years. (4) A 57-year-old postmenopausal woman had no known history of conjunctivitis, but her husband had

From the Nagashima Obstetrics-Gynecology Clinic.
Received for publication March 29, 1986; accepted July 13, 1986.
Reprint requests: Tetsuo Nagashima, M.D., Nagashima Obstetrics-Gynecology Clinic, 5-9-17 Kurihama, Yokosuka, Japan 239.

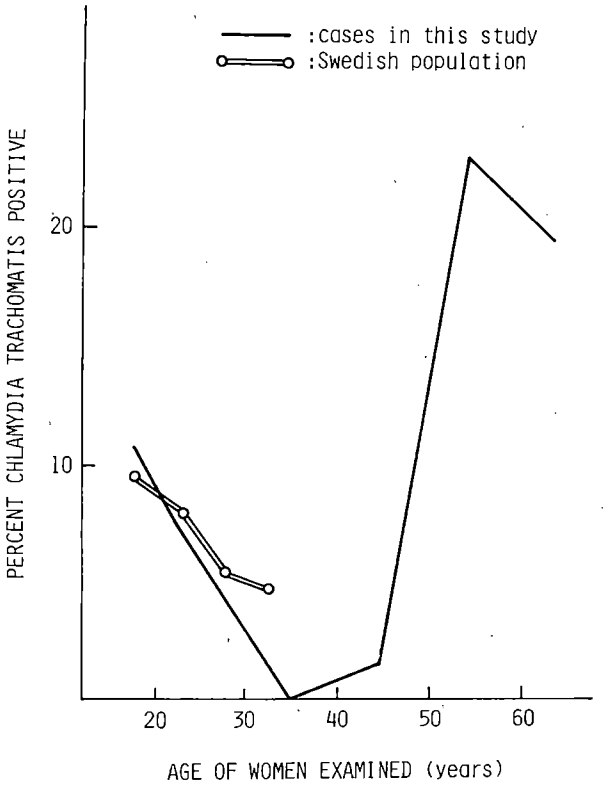


Fig. 1. Results of this study compared to those in the study of the Swedish population by Weström and Mårdh.[1]

history of trachomatous conjunctivitis premaritally. (5) Three other postmenopausal women had no known past or family history of any conjunctivitis.

Comment

Chlamydial cervicitis has long been thought to be most common in young, sexually active women. This study, however, shows a high incidence of asymptomatic chlamydial cervicitis in much older, postmenopausal women.

This finding is significant, since it demonstrates that chlamydial infections can be found in sections of the population in which it was thought such diseases were relatively uncommon.

Before the 1950s, when these subjects were younger, trachomatous conjunctivitis was quite common in Japan, but today it is rarely seen. On the other hand, inclusion conjunctivitis is still widely prevalent in Japan.

It has been established in the literature that the reticulate bodies of chlamydiae may reside intracellularly for long periods of time, protected from host antibodies by their lack of specific antigenicity.[2]

We therefore would like to suggest that the organisms survived within the women examined in this study for long periods of time, perhaps years.

The primary infections of *C. trachomatis* in women could have occurred through mucous membranes, such as in the eyes or elsewhere, with the organisms then surviving for decades before relocating to the uterine cervix through undetermined routes. Alternatively, the organisms could have resided within the cervical epithelia since the initial infections.

Additional studies will shed further light on the high prevalence of chlamydial cervicitis in postmenopausal women.

REFERENCES

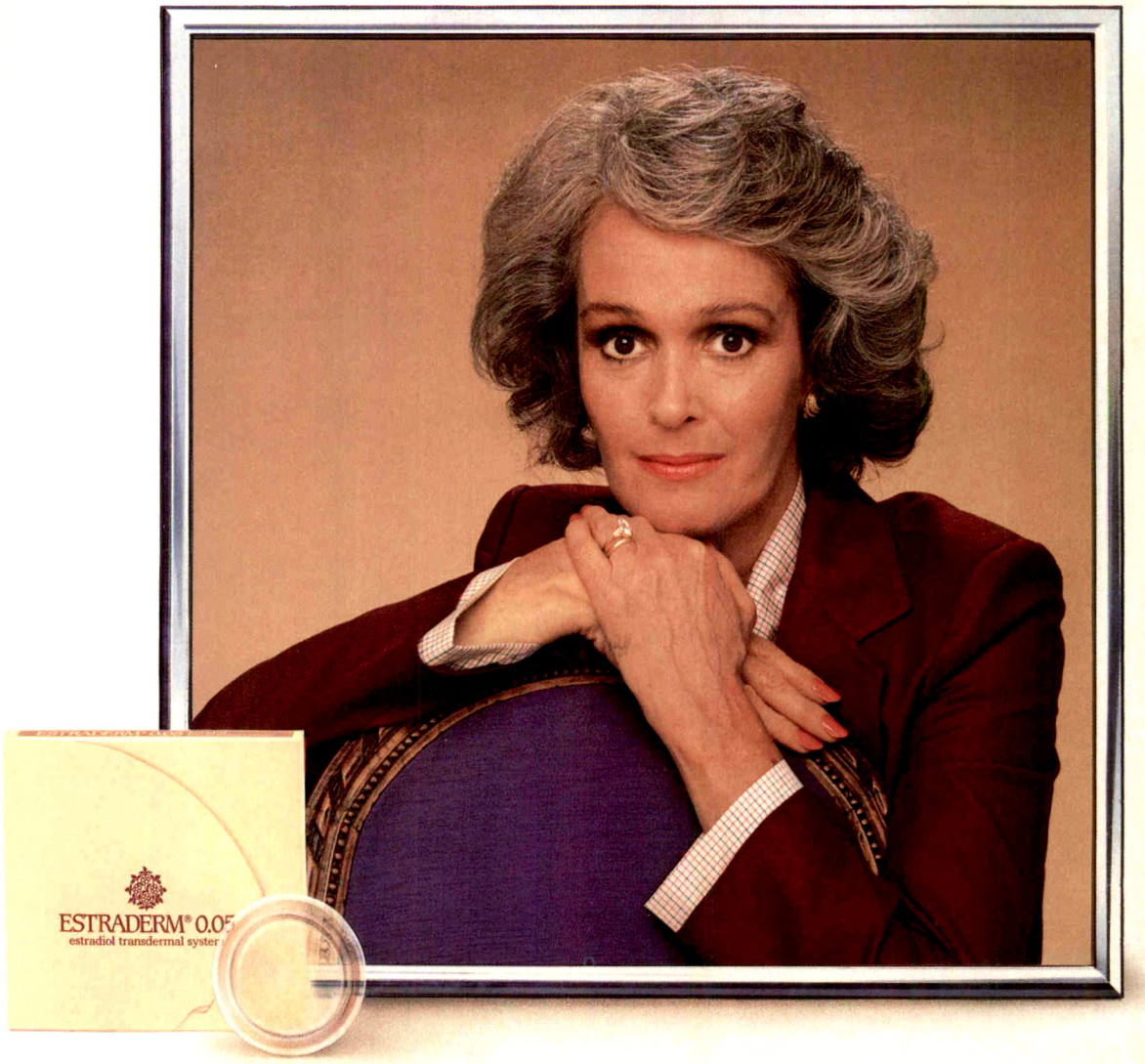
1. Weström L, Mårdh P-A. Genital chlamydial infections in the female. Amsterdam: Elsevier Biochemical Press, 1982: 121-39.
2. Manire GP. The Chlamydiae. In: Braude AI, Davis CE, Fierter J. Microbiology. Philadelphia: WB Saunders, 1982:518-20.



Now

The next generation of estrogen replacement therapy...

transdermal delivery

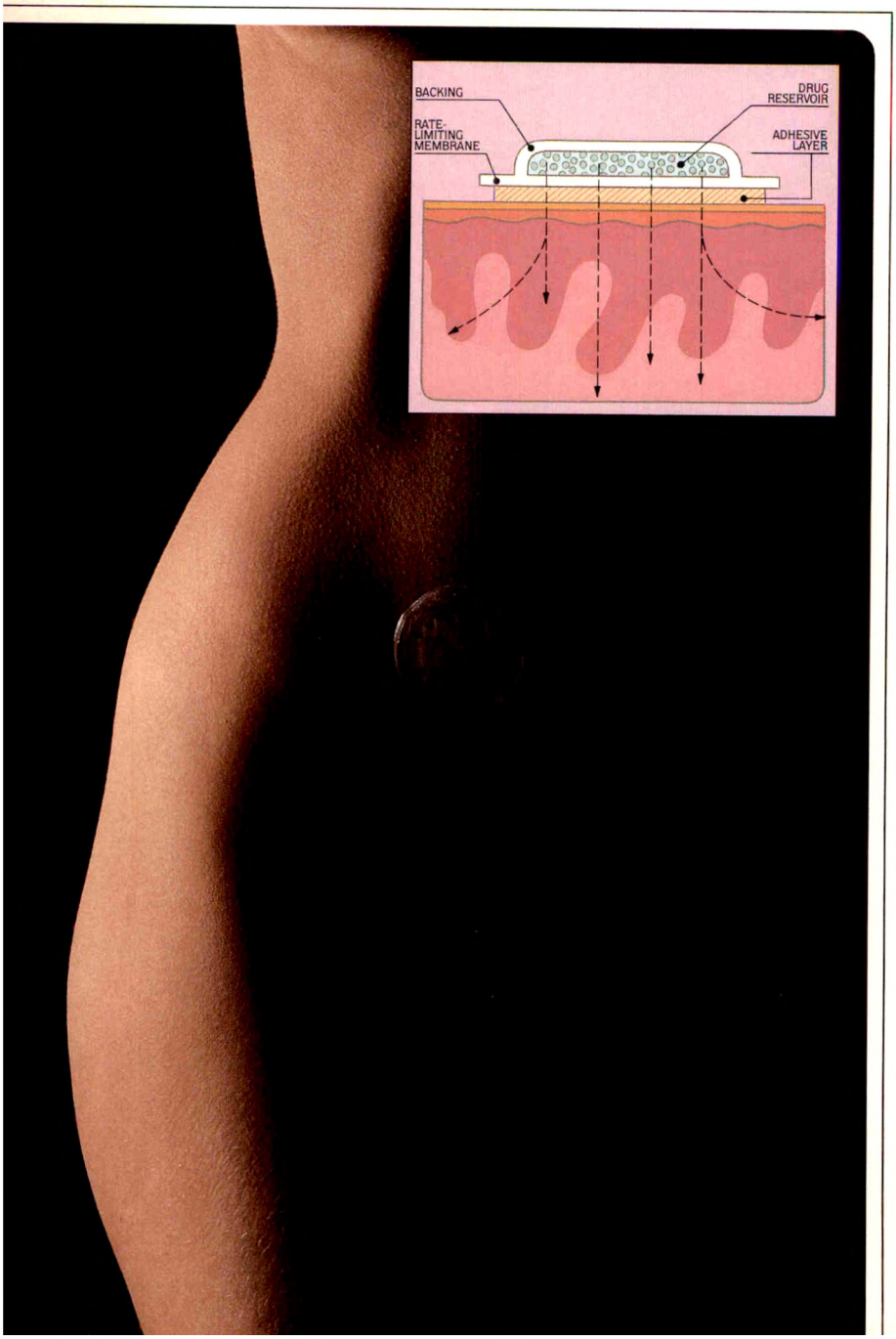


NEW

ESTRADERM®

estradiol transdermal system

Combines the advantages of transdermal delivery...



A specially designed transdermal delivery system

Providing the natural ovarian estrogen (17-beta estradiol) directly to the bloodstream and target organs; with no first-pass liver metabolism, permits use of extremely low dosage

Effectively controls estrogen deficiency symptoms

Steady-state relief of hot flashes, other menopausal symptoms associated with hot flashes, and vaginal discomfort

Well tolerated

Very low incidence of systemic side effects...few local problems of consequence

Easy to use; transdermal delivery preferred 3:1 by patients over tablets[1]

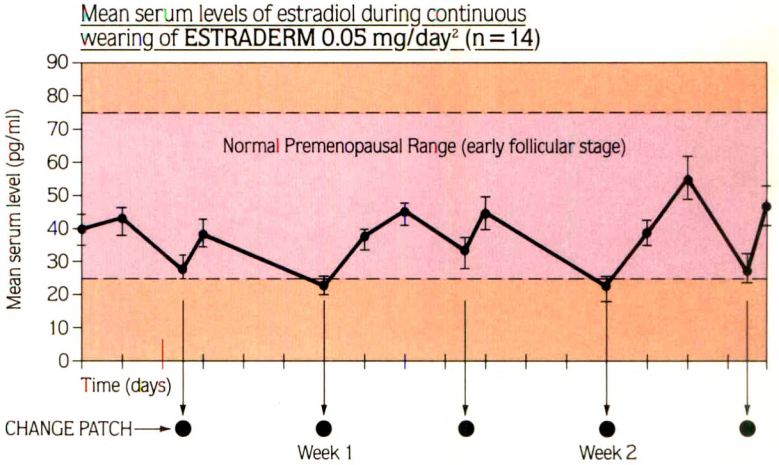
Usual starting dose: a 0.05-mg system changed twice weekly

C I B A

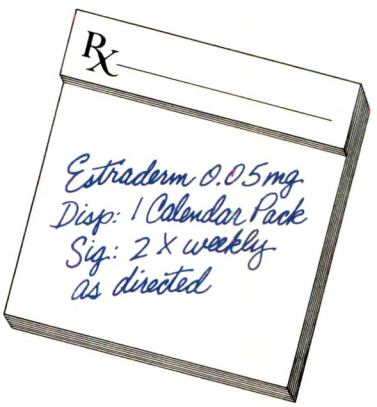
vith the benefits of
strogen replacement

estores and maintains premenopausal tradiol serum levels

stores estradiol levels to near-normal premenopausal ranges
chieves a physiologic 1:1 ratio of estradiol to estrone







ilable in dose-reminder calendar packs of eight
ches—0.05 mg/0.1 mg

I B A

ping today's women live healthier lives

References:

1. Data on file, CIBA Pharmaceutical Company.
2. Powers MS, et al: Pharmacokinetics and pharmacodynamics of transdermal dosage forms of 17 β-estradiol: Comparison with conventional oral estrogens used for hormone replacement. Am J Obstet Gynecol 1985; 152:1099–1106.

Please see next page
for Brief Summary of Prescribing Information.

ESTRADERM®

estradiol transdermal system

Continuous delivery for twice-weekly application.
BRIEF SUMMARY OF PRESCRIBING INFORMATION.
PLEASE SEE FULL PRESCRIBING INFORMATION.

ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than one year. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer-reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5-13.9 times greater than in nonusers. The risk appears to depend both on duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.

The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that women who had been exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than four per 1000 exposures. Furthermore, a high percentage of such exposed women (30-90%) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data on the use of other estrogens are not available, it cannot be presumed they would not induce similar changes.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb-reduction defects. One case control study estimated a 4.7-fold increased risk of limb-reduction defects in infants who had been exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses.

If Estraderm is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus and of the advisability of continuation of the pregnancy.

INDICATIONS AND USAGE

Estraderm is indicated for the treatment of the following: moderate-to-severe vasomotor symptoms associated with menopause; female hypogonadism; female castration; primary ovarian failure; and atrophic conditions caused by deficient endogenous estrogen production, such as atrophic vaginitis and kraurosis vulvae.

CONTRAINDICATIONS

Estrogens should not be used in women or men with any of the following conditions:

1. known or suspected cancer of the breast;
2. known or suspected estrogen-dependent neoplasia;
3. known or suspected pregnancy (see Boxed Warning);
4. undiagnosed abnormal genital bleeding;
5. active thrombophlebitis or thromboembolic disorders;
6. history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use.

WARNINGS

1. Induction of Malignant Neoplasms. Long-term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. There are now reports that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning.)

At the present time, there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of breast cancer, although a recent long-term follow-up of a single physician's practice has raised this possibility. Because of the animal data, there is a need for caution in prescribing estrogens for women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms.

2. Gallbladder Disease. A recent study has reported a two- to threefold increase in the risk of surgically confirmed gallbladder disease in postmenopausal women receiving oral estrogens, similar to the twofold increase previously noted in users of oral contraceptives.

3. Effects Similar to Those Caused by Estrogen-Progestogen Oral Contraceptives. There are several serious adverse effects of oral contraceptives and other high-dose oral estrogen treatments, most of which have not, up to now, been documented as consequences of postmenopausal estrogen replacement therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women.

a. Thromboembolic Disease. It is now well established that users of oral contraceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least four weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization.

While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found, this does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore, estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be used in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

b. Hepatic Adenoma. Benign hepatic adenomas have been associated with the use of oral contraceptives. Although benign and rare, these tumors may rupture and cause death from intra-abdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations, but they should be considered if abdominal pain and tenderness, abdominal mass, or hypovolemic shock occurs in patients receiving estrogen. Hepatocellular carcinoma has also been reported in women taking estrogen-containing oral contraceptives. The causal relationship of this malignancy to these drugs is not known.

c. Elevated Blood Pressure. Women using oral contraceptives sometimes experience increased blood pressure which, in most cases, returns to normal upon discontinuing the drug. There is now a report that this may occur with use of oral estrogens in the menopause, and blood pressure should be monitored with estrogen use, especially if high doses are used. Ethinyl estradiol and conjugated estrogens have been shown to increase renin substrate. In contrast to these oral estrogens, transdermally administered estradiol does not affect renin substrate.

d. Glucose Tolerance. A worsening of glucose tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen.

4. Hypercalcemia. Administration of high doses of estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases. If hypercalcemia occurs, use of the drug should be stopped and appropriate measures should be taken to reduce the serum calcium level.

PRECAUTIONS

General

1. A complete medical and family history should be taken before initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, as well as a cervical Papanicolaou test. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed.
2. Because estrogens may cause some degree of fluid retention, careful observation is required when conditions that might be influenced by this factor are present (e.g., asthma, epilepsy, migraine, and cardiac or renal dysfunction).
3. Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as uterine bleeding, mastodynia, etc.
4. Prolonged administration of unopposed estrogen therapy has been reported to increase the risk of endometrial hyperplasia in some patients. Estrogens should be used with caution in patients who have or have had endometriosis.
5. Oral contraceptives appear to be associated with an increased incidence of mental depression. Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.
6. Preexisting uterine leiomyomata may increase in size during prolonged estrogen use. If this occurs, estrogen therapy should be discontinued while the cause is investigated.
7. In patients with a history of jaundice during pregnancy, there is an increased risk that jaundice will recur with the use of estrogen-containing oral contraceptives. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated.
8. Estrogens may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients.
9. Because the prolonged use of estrogens influences the metabolism of calcium and phosphorus, estrogens should be used with caution in patients with metabolic bone diseases associated with hypercalcemia and in patients with renal insufficiency.

Information for Patients

See Patient Package Insert.

Drug/Laboratory Test Interactions

The results of certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The following changes have been observed with large doses of oral estrogen:

1. increased sulfobromophthalein retention;
2. increased prothrombin time; increased factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability;
3. increased thyroxine-binding globulin (TBG), leading to increased circulating total thyroid hormone (T_4) as measured by column or radioimmunoassay; free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered; TBG was not affected in clinical trials of Estraderm;
4. reduced response to the metyrapone test;
5. reduced serum folate concentration;
6. increased serum triglyceride and phospholipid concentration, and decreased pregnanediol excretion.

The pathologist should be informed that the patient is receiving estrogen therapy when relevant specimens are submitted.

Carcinogenesis, Mutagenesis, Impairment of Fertility

See **WARNINGS** and Boxed Warning.

Long-term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver.

Pregnancy Category X

See **CONTRAINDICATIONS** and Boxed Warning.

Estrogens should not be used during pregnancy.

Nursing Mothers

As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS

See **WARNINGS** and Boxed Warning regarding potential adverse effects on the fetus, induction of malignant neoplasms, increased incidence of gallbladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.

The most commonly reported adverse reaction to Estraderm in clinical trials was redness and irritation at the application site. This occurred in about 17% of the women treated and caused approximately 2% to discontinue therapy.

The following additional adverse reactions have been reported with estrogenic therapy, including oral contraceptives:

Genitourinary System: Breakthrough bleeding, spotting, change in menstrual flow; increase in size of uterine fibromyomata; change in cervical erosion and amount of cervical secretion.

Endocrine: Breast tenderness, breast enlargement.

Gastrointestinal: Nausea, vomiting; abdominal cramps, bloating; cholestatic jaundice have been observed with oral estrogen therapy.

Eyes: Steepening of corneal curvature; intolerance to contact lenses.

Central Nervous System: Headache, migraine, dizziness.

Miscellaneous: Change in weight, edema, change in libido.

HOW SUPPLIED

Estraderm 0.05 (estradiol transdermal system)—each 10 cm^2 system contains 4 mg of estradiol USP for nominal* delivery of 0.05 mg of estradiol per day

Patient Calendar Pack of 8 Systems — — — — — — — — — — — —NDC 0083-2310-08
Carton of 6 Patient Calendar Packs of 8 Systems — — — — — — — — — —NDC 0083-2310-62

Estraderm 0.1 (estradiol transdermal system)—each 20 cm^2 system contains 8 mg of estradiol USP for nominal* delivery of 0.1 mg of estradiol per day

Patient Calendar Pack of 8 Systems — — — — — — — — — — — —NDC 0083-2320-08
Carton of 6 Patient Calendar Packs of 8 Systems — — — — — — — — — —NDC 0083-2320-62

*In vivo delivery of 0.05 or 0.1 mg of estradiol per day via skin of average permeability (interindividual variation in skin permeability is approximately 20%).

Do not store above 86°F (30°C).
Do not store unpouched. Apply immediately upon removal from the protective pouch.

CAUTION: Federal law prohibits dispensing without prescription.

C I B A

CIBA Pharmaceutical Company, Division of CIBA-GEIGY Corporation
Summit, New Jersey 07901

C86-55 (Rev. 10/86)

© 1986, CIBA.

Printed in U.S.A. 109-8236-A

Alcohol teratogenicity in the human: A detailed assessment of specificity, critical period, and threshold

Claire B. Ernhart, Ph.D., Robert J. Sokol, M.D., Susan Martier, M.S.S.A., Pamela Moron, B.S., Debra Nadler, B.S., Joel W. Ager, Ph.D., and Abraham Wolf, Ph.D.
Cleveland, Ohio, and Detroit, Michigan

Though the occurrence of alcohol-related birth defects is well documented in the human and in animal models, definition of specificity, critical period, and dose-response threshold with a precision adequate for clinical risk assessment and management has been lacking. Data from a cohort of 359 neonates, from a large prospective observational study in which chronic alcohol problems and maternal drinking were assessed during pregnancy and standardized neonatal examinations were blinded for prenatal information, were analyzed with the use of multivariate techniques, with uniform control for confounding by eight factors. Craniofacial abnormalities were found to be definitively related to prenatal alcohol exposure in a dose-response manner ($p < 0.001$); a significant, but less striking, relationship was observed for other anomalies ($p < 0.01$). The critical period for alcohol teratogenicity was confirmed to be around the time of conception. Risk for anatomic abnormalities in the offspring was clearly defined among the 5.6% of infants whose mothers drank more than three ounces of absolute alcohol, that is, more than six drinks, per day. Because of a trend toward an increase in craniofacial abnormalities with increasing embryonic alcohol exposure at lower levels, a clear threshold could not be defined. These are not experimental data and the results should not be overinterpreted. Nevertheless, pending further studies with larger samples, the findings suggest that to completely avoid alcohol-related anatomic abnormalities, advice to discontinue drinking or at least to reduce it to a minimal level before conception is clinically appropriate. (AM J OBSTET GYNECOL 1987;156:33-9.)

Key words: Alcohol-related birth defects, fetal alcohol effects, critical period, neonatal anomalies

That alcohol is a potent teratogen in the human and in several animal models has been well established in a large number of studies published during the last 13 years.[1] Indeed it has been estimated that up to 5% of anatomic congenital anomalies may be attributable to prenatal alcohol exposure.[2] Although the diagnosis of the full fetal alcohol syndrome requires the presence of prenatal and/or postnatal growth retardation, central nervous system involvement, and the presence of characteristic facial dysmorphology,[3] it has been hypothesized that lesser effects, including isolated congenital defects, may be associated with lower, less frequent and/or differently timed doses of alcohol.

From the Department of Psychiatry, Case Western Reserve University and Cleveland Metropolitan General Hospital, and the Department of Obstetrics and Gynecology, Wayne State University/Hutzel Hospital.
This research was supported in part by National Institute of Child Health and Human Development Grant No. HD14883, by Grant No. 12-69 from the March of Dimes Birth Defects Foundation, by National Institute of Alcohol Abuse and Alcoholism Grant AA06571 (to C. B. E.), and by National Institute of Alcohol Abuse and Alcoholism Grant AA03282 (to R. J. S.).
Received for publication May 12, 1986; revised June 9, 1986; accepted August 11, 1986.
Reprint requests: Claire B. Ernhart, Ph.D., Department of Psychiatry, Cleveland Metropolitan General Hospital, 3395 Scranton Road, Cleveland, OH 44109.

Defining alcohol teratogenicity in the human with a precision desirable for offering patients rational advice and for clinical risk assessment and management has been difficult. First, the drinking of alcoholic beverages is a complex human behavior; it is difficult to accurately ascertain historically and difficult to represent quantitatively.[1, 3] Second, the pattern of alcohol-related birth defects in individuals is not invariant. The list of features observed is lengthy[4] and no single feature is noted in every case. Few reports describe the frequency of each. One exception is an early article by Hanson et al.,[5] which included a tabulation of the occurrence of nine physical anomalies recorded for 41 children with fetal alcohol syndrome. The most frequently noted feature, short palpebral fissures, was observed in 92% of the children. No other feature was reported for more than 65% of the sample.

Our research group has been studying alcohol-related risks in human pregnancy for several years. In a previous report,[6] we described a neonatal examination for anomalies, which was based on the descriptions of fetal alcohol syndrome by Hanson et al.[5] and others. A tally of detected anatomic abnormalities was found to be related significantly to maternal alcohol abuse, as measured by the Michigan Alcoholism Screening Test

(MAST).[7] In the MAST-positive group a dose-response relationship between first-trimester alcohol exposure and the tally was obtained. While maternal alcohol use was related to neonatal size (another of the reported sequelae of fetal alcohol exposure), this effect was attenuated with control of parental size measures. Neonatal behavior scores were unrelated to the MAST classification and to maternal alcohol use. Thus the anomalies tally appeared to be more sensitive to prenatal alcohol exposure than either infant size or behavioral measures.

To define alcohol teratogenicity in the human as precisely as possible, the present report extends our previous findings by attempting to answer three questions concerning the association of fetal alcohol exposure and neonatal anomalies. First, are craniofacial anomalies more specifically related to alcohol exposure early in gestation than other anomalies? Second, can a critical period for alcohol-related dysmorphology be identified? Third, can a threshold for alcohol-related teratogenicity be discerned?

Background and rationale

Specificity of craniofacial anomalies. Two issues bear on the decision to differentiate craniofacial anomalies from other anomalies in the analyses to be presented. First, an analysis demonstrating a strong and rather specific alcohol exposure relationship with craniofacial features would strengthen the inference that the abnormalities observed are alcohol-related. Second, given that individuals differ in their vulnerability to alcohol exposure, the focus on craniofacial anomalies might provide us with a neonatal indicator of later developmental deficit.

The possibility that abnormalities apparently related to fetal alcohol exposure might be attributable to confounding conditions, such as smoking, use of other drugs, and poor nutrition, is difficult to rule out in studies in humans.[1, 2] Given the limitations of the assessment of such conditions and the interrelatedness of such variables in epidemiologic studies, control through statistical methods often underadjusts. The attribution of effect to alcohol exposure, however, would be enhanced given that the effect is stronger for those anomalies that are most distinctive and which have been specifically linked to fetal alcohol exposure, i.e., the craniofacial anomalies.

Craniofacial anomalies observed in the neonatal period may provide a powerful indicator of later functioning level, because of a possible association with functional deficits of the sensory organs or with abnormal growth of the brain.[8, 9] We plan to examine this important perinatal developmental issue, with medicological implications, in a later study of this cohort of children.

The Anomalies Tally included both craniofacial anomalies and various cardiac, muscular, and skeletal features described in various reports of fetal alcohol syndrome. The specific hypothesis in the current study was that the effect of alcohol exposure would be more decisive in the group of craniofacial anomalies than in the group of other anomalies.

Critical period. The timing of prenatal exposure to alcohol would be expected to be a major determinant of the adverse effects observed. Thus alcohol-related intrauterine growth retardation[10] and disruption of the normal appearance of amniotic fluid phospholipids[11] have been shown to be related to continued maternal drinking in the third trimester; neither fetal alcohol effect was observed in pregnancies in which the mother became abstinent before that period, with clear implications for prevention.[1]

An issue of considerable clinical as well as theoretical importance concerns the specific time during pregnancy of greatest vulnerability to alcohol teratogenicity. It is generally recognized that the first trimester, in particular, the period from 4 to 10 weeks' gestation (that is, from 2 to 8 conceptual weeks, which is the time of embryogenesis), is most critical for the induction of anatomic abnormalities. It was for this reason that our initial analyses assessed the effects of first-trimester exposure for this outcome measure.[6] However, in our previous study, data concerning early pregnancy exposure were based on maternal drinking data from a mean of approximately 12 weeks' gestation. We now contrast this time of exposure with an index heavily weighted with second-trimester exposure and, most importantly, an index that is an estimate of embryonic alcohol use.

Threshold. In our previous report of the relationship of anomalies and alcohol use among women with positive MAST results, an augmented effect for the women reporting the highest alcohol use was noted.[6] In the present report, we attempt to identify a threshold of effect for alcohol-related neonatal anomalies. Identification of a threshold, if present, for this apparently very sensitive fetal alcohol effect would be most useful clinically in counseling patients concerning alcohol risk to their offspring.

Methods

A description of the sample and the methods of collecting the data has been reported previously[12] and a summary is presented here. This study was approved by the human investigation committee of the institution.

Sample. The infants in this study were the offspring of disadvantaged urban women who were screened at their first antenatal visit for chronic alcohol problems. The instrument used was the MAST.[7] This is a well-studied, widely used, 25-question instrument, focusing on alcohol-related psychosocial disruption, for the de-

tection of chronic alcohol problems, that is, alcohol abuse and alcohol dependence. Evidence of the validity of the MAST for the present study comes from research describing an earlier sample of 2913 women from the same population.[12] In this report, MAST-positive pregnant women were found to be 8.3 times more likely to have reported drinking ≥1 ounce of absolute alcohol per day, 19 times more likely to have reported drinking ≥2 ounces of absolute alcohol per day, and 17.5 times more likely to have reported drinking ≥3 ounces of absolute alcohol per day than women with negative MAST results. The score can range from 0 to 53, with a score of ≥5 considered positive.

The MAST was administered to all gravid women presenting for antenatal care in the clinic setting of a hospital that serves primarily disadvantaged patients. (About 80% to 90% of hospital patients are in Social Classes IV and V, Hollingshead Two Factor Index. Among those using the clinic this is close to 100%.) From this population all MAST-positive women and matched control subjects (MAST negative) were entered in a study of the use of alcohol in pregnancy extending over a period of several years.[12] The matching variables were race, smoking, parity, date of recruitment, drug abuse, prepregnancy weight, and weeks' gestation at registration. From this study of matched MAST-positive and MAST-negative gravid women studied through pregnancy, 543 deliveries occurred within a specified 15-month period. These mother-infant pairs were reviewed for inclusion in a cohort to be followed up through the child's preschool years for the evaluation of alcohol-related birth defects. Infants were excluded from the prospective study as follows: 87 for health-related reasons (gestation <37 weeks, indications for neonatal intensive care, maternal psychosis, and maternal narcotics use), 47 because of parental refusal, 16 because of primary language other than English, 14 because they were missed by the research staff (weekend delivery and discharge), 10 because of plans to move from the area, and 10 because of adoption. The cohort included 359 mother-infant pairs. Of these, 113 were black and 246 were white. In order to avoid the exclusion of yoked cases, one-to-one matching was not retained for the neonatal or prospective study.

Examination. Each neonate was examined by a trained research nurse who was blinded to the individual histories of prenatal alcohol exposure. A listing of anomalies observed in one or more of the 359 children of the study is provided in Table I. The tallies ranged from 0 to 11, with 26 (7.2%) of the 359 neonates presenting no tallied item. Considered individually, most items of the Anomalies Tally are neither very unusual nor very worrisome. The rationale behind the development of the Anomalies Tally is that, as the number of these increases, the results of the examination become more characteristic of the pattern of dysmorphology identified with fetal alcohol syndrome.

The items of the Anomalies Tally observed among the children of the study were divided into those of the

Table I. Incidence of anomalies by MAST group

	MAST-positive (n = 176)		*MAST-negative (n = 183)*
Craniofacial anomalies			
Eyes			
Epicanthal folds	7		2
Short palpebral fissures	31		26
Nose			
Short	48		40
Up-turned	42		38
Broad, deep bridge	97		92
Hypoplastic	4		0
Ears			
Posterior rotation	60		36
Simply formed	46		34
Odd shape	1		0
Maxilla			
Hypoplastic philtrum	24		17
Mandible			
Micrognathia	19		17
Lips			
Thin upper vermilion	48		33
Head			
Macrocephaly	0		1
Odd shape	0		1
Face			
Asymmetrical	1		0
Mean	2.4		1.8
SD	1.5		1.4
t Test		3.85*	
Other anomalies			
Cardiac			
Murmurs, nonfunctional	22		15
Cutaneous			
Hirsutism	69		66
Hemangiomas	0		1
Accessory nipples	4		1
Nail hypoplasia, esp. 5th	11		9
Abnormal palmar crease	9		6
Renal-genital			
Labial hypoplasia	2		2
Hypospadias	1		0
Hydrocele	1		0
Skeletal			
Pectus excavatum	3		2
Limited joint movement (esp. fingers, elbows)	0		1
Hip dislocation	1		0
Supernumerary fingers	1		1
Clubfoot	1		0
Muscular			
Hernias of umbilicus	4		6
Diastasis recti	33		22
Mean	0.9		0.7
SD	0.9		0.8
t Test		2.24†	

*p < 0.001.
†p < 0.05.

Table II. The relation of first-trimester alcohol exposure to craniofacial and other anomalies among infants of MAST-positive women

Anomalies index	*Alcohol exposure index*	*Covariate (% of variance)*	*Increment (% of variance)*	*F*	*p*
Craniofacial	Absolute alcohol per day, first trimester	12.0	3.6	6.88	0.01
	Proportion/drinking days	12.0	4.9	9.52	0.002
Other	Absolute alcohol per day, first trimester	8.3	0.5	0.94	0.33
	Proportion/drinking days	8.3	0.8	1.38	0.24

The variance for the missing data variables is not included in either the covariate or exposure-increment variance.

craniofacial area and those of a more general nature. This division and the frequency of each item in the MAST-positive and MAST-negative groups are also indicated in Table I. Craniofacial items not noted in the cohort were ptosis, cleft palate, and cleft lip. Among the other anomalies listed for examination, inguinal hernia was not recorded for any infant in the study.

Indices of alcohol exposure. The first indicator suggesting fetal alcohol exposure was the initial selection criterion, positive or negative MAST classification. While some MAST-positive women described themselves as being abstinent during pregnancy, MAST-positive women averaged moderately high levels of alcohol consumption.[12] Furthermore, as previously reported,[6] all women in the highest category of first-trimester alcohol use were MAST positive. This association of the MAST classification and alcohol use further enhances the validity of the MAST for use with pregnant women.

In addition to the administration of the MAST at the first antenatal visit, carefully structured interviews[13] were used at each antenatal visit to elicit a recall of alcohol use on a day-by-day basis for beer, wine, and liquor. The major indices of alcohol use were based on ounces of absolute alcohol per day. The median and modal point in pregnancy for the first interview was 19 weeks and the index computed for the data of this visit is designated as "absolute alcohol per day, second trimester," in the analyses that follow.

Of the 359 participants, 139 had enrolled before the end of the first trimester; all available first-trimester sampled days were used to compute "first-trimester absolute alcohol per day" for these cases. The availability of first-trimester data was not related significantly to the respective outcome measures. In the regression analyses that follow, the lack of data for the remaining cases was handled through the use of a missing data variable.[14] The use of the missing data variable in these analyses served both to maintain the statistical power of the analyses and to provide a test of any bias that might be introduced by the sampling limitations. The tests for bias were negative in each analysis; that is, the respective indices for neonatal anomalies did not differ as a function of whether or not the mother enrolled for care in the first trimester.

The third index of alcohol exposure is designated as "embryonic absolute alcohol per day." A note of explanation is in order. Neither measures of alcohol use at or about the time of conception nor measures of prepregnancy drinking were included in the original protocol. Since the period shortly after conception is the period of embryogenesis and possibly the period of greatest risk for alcohol-related dysmorphology, we sought a method of using the available data (MAST score and measures of alcohol use during pregnancy) to provide estimates for each individual of alcohol consumption at that period.

The method by which this index was developed has been reported previously.[15] In brief, in a separate sample of 57 intensively studied pregnant women in a different city, at a median gestational duration of 19 weeks, women were interviewed with respect to their alcohol intake during the previous 2 weeks. The interview protocol, including the administration of the MAST, followed closely the carefully structured and nonthreatening techniques used originally in the present study.[13] (The structure of the interview asks for recall of amounts and kinds of beverage by day of the week. Reference to relevant social and entertainment activities is used to aid recall by days.) The women were then questioned in a similar manner about their usual prepregnancy drinking and about their drinking at the time at which they thought they conceived. In this interview the prepregnancy and current drinking estimates provided structure for the questions regarding drinking about the time of conception.

Multiple regression analysis of the data of these 57 women, with MAST score and current drinking (absolute alcohol per day) used as independent variables and embryonic drinking used as the dependent variable, yielded the following equation: embryonic absolute alcohol per day = (0.12 × MAST score) + (0.67 × current absolute alcohol per day) + 0.08. (R^2 = 37.1%.) This equation was executed for each pregnancy in the current sample. The resulting estimate was used as an index of embryonic alcohol exposure.

One additional index used in the specificity analyses was proportion of drinking days in the first trimester.

Table III. The relationship of congenital anomalies to absolute alcohol per day in three periods of exposure

Period of exposure	*Anomalies*	*Covariate (% of variance)*	*Increment (% of variance)*	*F*	*p*
Second trimester	Craniofacial	8.5	0.7	2.64	0.11
	Other	8.7	0.4	1.51	0.22
First trimester*	Craniofacial	8.5	1.3	4.79	0.03
	Other	8.7	0.7	2.48	0.12
Embryonic	Craniofacial	8.5	3.4	13.46	0.000
	Other	8.7	2.4	9.29	0.002

*A missing data variable was used in first-trimester analyses. The missing data variance is not included in either the covariate or the exposure-increment variance.

This index of persistence or regularity of maternal drinking was computed as the ratio of days on which drinking occurred to the number of days covered in the interviews.

Methods of data analysis. Initial analyses used *t* tests to contrast MAST-positive and MAST-negative groups. These provide only a preliminary indication of the relations involved, since they did not control for confounding variables. Eight covariates were selected a priori as possible confounders. These were maternal age, race, parity, nutrition, cigarette smoking, the use of marijuana, and the use of other illicit drugs during pregnancy. Demographic data were obtained at the first antenatal visit. Other data (aside from infant sex) were collected in interviews during the course of pregnancy. We have previously reported the association of MAST classification and nutritional measures.[12] We have also previously noted an association of race, maternal age, and parity and the use of illicit drugs with a full tally of anomalies (both craniofacial and other).[6] In order to maintain consistency between analyses, we did not eliminate the nonsignificant covariates in the analyses but used the full set in each statistical model.

The statistical method used for all but the initial analyses was incremental multiple regression with the independent variable under test entered after the full set of covariates.[14] Because the absolute alcohol per day indices were positively skewed, logarithmic transformations were used.

Because of multiple comparisons made in the study, $0.05 < p < 0.01$ was considered borderline and $p < 0.01$, significant.

Results

Specificity. Table I includes *t* tests substantiating significant differences between the MAST-positive and MAST-negative groups for the tabulations of craniofacial and other anomalies. These were further tested in regression analyses with MAST as the independent variable, control of the covariates, and the two tallies as dependent variables. For the craniofacial tally the initial variance of the model (associated with covariates) was 8.5% and the variance increment associated with the MAST classification was 2.6% ($p = 0.002$). For the other anomalies, the initial variance was 8.7% and the variance increment for the MAST was 1.0% ($p = 0.06$). For both tallies the covariate contributing most strongly to the models was race.

Because a much wider range of alcohol use was reported by MAST-positive women, further tests of the specificity of the craniofacial anomalies were made separately for the infants of MAST-positive and MAST-negative women. As shown in Table II, among infants from MAST-positive pregnancies, the count of craniofacial anomalies was related significantly both to the first-trimester absolute alcohol per day and to the proportion of drinking days; corresponding analyses for members of the MAST-negative group failed to reach statistical significance. In both groups the variance in other anomalies accounted for by the first-trimester alcohol indices was small and nonsignificant.

Critical period. Data for the entire sample ($n = 359$) were used for comparisons of the three periods of exposure for each of the two measures of congenital anomalies. As shown in Table III, neither of the counts of congenital anomalies was related significantly to absolute alcohol per day, second trimester, and only the count of craniofacial anomalies was related significantly to absolute alcohol per day, first trimester. Embryonic absolute alcohol per day explained considerably more variance in both the craniofacial and the other anomalies than either late first-trimester or second-trimester absolute alcohol per day indices.

Threshold. The relationship of each group of anomalies, residualized for the eight covariates, to the embryonic alcohol index is shown in Fig. 1. The craniofacial anomalies were related in a consistent dose-response manner to maternal drinking in the period of embryonic development. In spite of one slightly deviant point, the plot for other anomalies was generally consistent with the expected dose-response pattern. The graphed data suggest the possibility of a nonlinear fit, that is, that the effect was due primarily to the peak incidence at absolute alcohol per day >3.0. This pos-

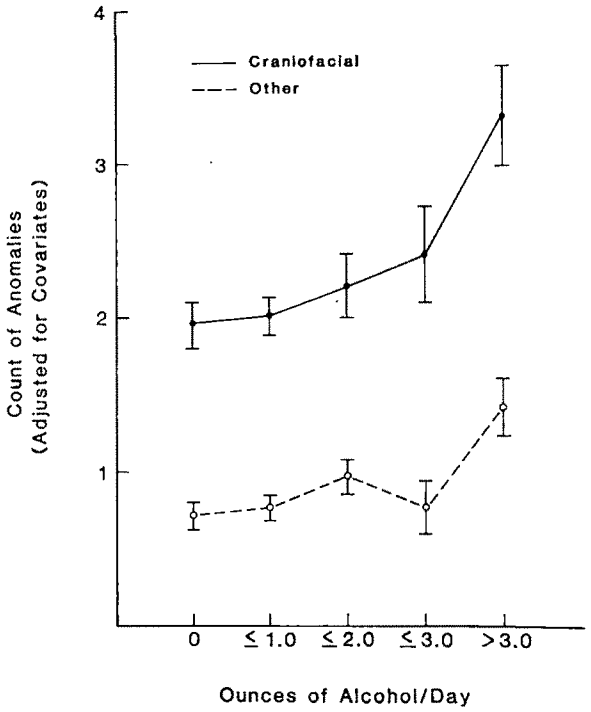


Fig. 1. Means (+SE) of craniofacial and other anomalies, both residualized for eight covariates, as a function of embryonic absolute alcohol per day in ounces. For absolute alcohol per day = 0, n = 95; for <1.0, n = 161; for <2.0, n = 60; for <3.0, n = 23; and for >3.0, n = 20. F and p values are shown in Table III.

sibility was tested in two ways. The first, a polynomial analysis with inclusion of a quadratic term, was not significant for either anomalies measure; the hypothesis of a nonlinear relationship was not supported. The possibility of a threshold of effect was also explored through truncation of the 20 cases (5.6% of the sample) with absolute alcohol per day >3.0. Under truncation, the effect sizes for the craniofacial and other anomalies were reduced to 1.6% and 0.9%, respectively. For the craniofacial anomalies, this remaining incremental variance was of borderline statistical significance (p = 0.017).

Comment

The key findings of this study are that anatomic abnormalities detectable in the neonate, particularly of the craniofacial area, are related in a clear dose-response manner to prenatal alcohol exposure and that, as would be expected embryologically, the critical period is in the early first trimester, that is, the embryonic period. Though there has been convincing evidence for a dose-response relationship between prenatal alcohol exposure and a range of adverse outcomes in animal models[1] and evidence in the human for a dose-response relationship to risk for small for gestational age birth, to the best of our knowledge, the current findings are the first to define a dose-response relationship between embryonic exposure to alcohol and a specific set of anatomic congenital abnormalities in the human.

These findings must not be overinterpreted. These are not experimental data. Although neonatal examinations were blinded for all prenatal information, including maternal drinking history,[12] and we have been unable to detect bias in these examinations, despite extensive analyses, there is still a possibility of confounding in observational data. Statistical control, often incomplete, here for eight covariates, may or may not have been entirely adequate. It is possible, for example, that the nutrition measures (24-hour recall for Recommended Dietary Allowances in five food categories) may not have been sufficiently sensitive to alcohol-related nutritional deficits and that such deficits are the immediate antecedents of the anomalies observed. Data consistent with this interpretation would not eliminate alcohol in the chain of causation, but they would describe the causal pathway more specifically than is the case with the present data set. Such considerations cannot be dismissed completely in epidemiologic analyses such as this. Nevertheless, aside from a few very rare conditions, such as fetal hydantoin syndrome, the pattern of anomalies associated with fetal alcohol syndrome appears from the current analysis, as well as clinically, to be reasonably specific to the infant exposed to alcohol prenatally.

An additional caveat relates to the finding that even with statistical control of covariates, the association of MAST classification and craniofacial abnormalities remained strong and highly significant. The MAST measures chronic alcohol-related problems, so this finding is not inconsistent with the inference that maternal physiologic change secondary to alcohol dependence-abuse might contribute to abnormal infant outcome, as suggested by Majewski and Goecke.[4] Alternatively, we have previously shown that drinking near the time of conception, the period of maximal vulnerability for anatomic defects, is nearly as heavy as that in the nonpregnant state.[15] Because of the strong correlation between MAST and absolute alcohol per day, whether it is the alcohol, the alcoholism, or both, so to speak, cannot be determined from these data. Results from animal studies, however, suggest that the major effect results from direct exposure of the embryo to alcohol.

Two of the aims of the study, the greater specification of craniofacial anomalies and the identification of a critical period for exposure, are defined clearly in the findings detailed in this report. The further use of the data in a search for a threshold of effect was less definitive. We are very confident in concluding that the consumption of more than 3 ounces of absolute alcohol per day, that is, six cans of beer, glasses of wine, or

mixed drinks per day, at or about the time of conception greatly increases the risk for alcohol teratogenicity. A "no effect zone" often seen with teratogens might be expected. Indeed, some studies, for instance, that of Lumley et al.,[16] have not detected an alcohol effect at low levels of intake. However, in the current sample, truncation to retain only those subjects consuming less alcohol reduced, but did not wholly eliminate, this effect. This reduction in the effect and the curvilinear trend presented in Fig. 1 suggest that a threshold level might be observed with a larger database, but the trend toward an effect at lower doses suggests a clinical implication. Prudence supports the recommendation that to completely avoid alcohol-related anatomic abnormalities, women who are planning pregnancy should avoid alcohol consumption or, at least, reduce their consumption to a minimal level.

We are grateful for the assistance provided by Sheldon Miller, M.D., Mary J. Kennard, R.N., C.P.N.P., and Nancy Golden, M.D.

REFERENCES

1. Sokol RJ. The effects of alcohol on pregnancy outcome. Alcohol Health Res World 1984;9:27-68.
2. Sokol RJ. Alcohol and abnormal outcomes of pregnancy. Can Med Assoc J 1981;125:143-8.
3. Rosett HL. A clinical perspective on the fetal alcohol syndrome [Editorial]. Alcoholism Clin Exp Res 1980;4: 119-22.
4. Majewski F, Goecke TO. Alcohol embryopathy. In: Abel EA, ed. Fetal alcohol syndrome. Boca Raton, Florida: CRC Press, 1982 vol 2:65-88.
5. Hanson JW, Jones KL, Smith DW. Fetal alcohol syndrome. JAMA 1976;235:1458-60.
6. Ernhart CB, Wolf AW, Linn PL, Sokol RJ, Kennard MJ, Filipovich HF. Alcohol-related birth defects: syndromal anomalies, intrauterine growth retardation, and neonatal behavioral assessment. Alcoholism Clin Exp Res 1985; 9:447-53.
7. Selzer ML. The Michigan Alcoholism Screening Test: the quest for a new diagnostic instrument. Am J Psychiatry 1971;127:1653-8.
8. Streissguth AP, Herman CS, Smith DW. Stability of intelligence in the fetal alcohol syndrome: a preliminary report. Alcoholism Clin Exp Res 1978;2:165-70.
9. Majewski F. Alcohol embryopathy: some facts and speculations about pathogenesis. Neurobehav Toxicol Teratol 1981;3:129-44.
10. Rosett HL, Weiner L, Lee A, Zuckerman B, Dooling E, Oppenheimer E. Patterns of alcohol consumption and fetal development. Obstet Gynecol 1983;61:539-46.
11. Halvorsen PR, Gross TL, Sokol RJ. Effect of heavy alcohol intake on amniotic fluid phospholipids in late pregnancy. Am J Perinatol 1985;2:173-7.
12. Sokol RJ, Miller SI, Debanne S, et al. The Cleveland NIAAA prospective alcohol-in-pregnancy study: the first year. Neurobehav Toxicol Teratol 1981;3:203-9.
13. Sokol RJ, Martier S, Ernhart CB. Identification of alcohol abuse in the prenatal clinic. In: Early identification of alcohol abuse. Bethesda, Maryland: National Institute on Alcohol Abuse and Alcoholism, 1985. (National Institute on Alcohol Abuse and Alcoholism Research Monograph; no. 17).
14. Cohen J, Cohen P, ed. Applied multiple regression/correlation analysis for the behavioral sciences. 2nd ed. Hillsdale, New Jersey: Lawrence Erlbaum, 1983:275-89.
15. Moron P, Nadler D, Martier S, Sokol RJ. Estimating embryonic alcohol exposure [Abstract]. Alcoholism Clin Exp Res 1985;9:197.
16. Lumley J, Correy JF, Newman NM, Curran JT. Cigarette smoking, alcohol consumption and fetal outcome in Tasmania 1981-82. Aust NZ J Obstet Gynaecol 1985;25: 33-5.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1987 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 156 (January-June) and Vol. 157 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Abruptio placentae: Clinical management in nonacute cases

John S. Sholl, M.D.
Evanston, Illinois

One hundred thirty cases of clinically diagnosed abruptio placentae encompassing the wide range of acuity were grouped by gestational age at delivery into previable, preterm, and term divisions for comparison of demographic data, presenting symptoms, delay to delivery, mode of delivery, and delivery indications. Attention was focused on the preterm group of patients to assess the implications of presenting symptoms, the usefulness of ultrasonography, and the safety and efficacy of tocolysis. Cigarette smoking and a previous poor obstetric history were found to be more frequent in the preterm compared to the term abruptio placentae. Ultrasonic visualization of a clot was successful in 25% of the preterm patients but otherwise appeared to have little or no impact on course or management. Tocolysis for the preterm patients appeared to be beneficial in prolonging gestation and did not increase the likelihood of cesarean delivery, hemorrhage, or fetal distress. The cumulative rates of delivery following admission were compared with and without tocolysis, with and without sonographic visualization of a clot, and overall as a function of gestational age at initial hospitalization. Perinatal mortality was 17%. Inpatient management with frequent fetal heart rate monitoring, tocolysis if indicated, and timely use of cesarean delivery are advocated to promote prolongation of the pregnancy and minimized perinatal mortality. (AM J OBSTET GYNECOL 1987;156:40-51.)

Key words: Abruptio placentae, ultrasonography, tocolysis

Bleeding in the third trimester of pregnancy has been reported to occur in approximately 4% of pregnancies. Among these episodes about 20% can be attributed to placenta previa and 30% to definitive abruptio placentae.[1] Thus about half of the bleeding episodes in pregnancy remain ill-defined as small or "marginal" placental separations (sometimes associated with labor), as local birth canal lesions, or as bleeding with no determinable etiology. Heretofore, attention has been focused mostly on abruptio placentae of significant consequence, that is, grades 2 or 3 in the classification system first proposed by Page et al.[2] (Table I), with maternal hemodynamic instability, coagulopathy, fetal distress or death,[3-6] or exclusively toward cases of abruptio placentae that have resulted in fetal death.[7] A number of authors have concentrated on the risk factors associated with such major cases, which have included hypertension, multiparity, cigarette smoking, previous abruptio placentae, and occasionally trauma.[7-9] With these cases of acute abruptio placentae the need for rapid diagnosis and treatment and the significant impact of cesarean delivery in reducing perinatal mortality have been emphasized.[4, 5, 10, 11]

"Marginal sinus separation," "chronic abruption," or "limited abruption" are nonstandardized terms to categorize bleeding that has a more prolonged and/or perhaps transiently noncatastrophic course when placenta previa, birth canal injury, and major abruptio placentae, that is, grades 2 or 3, are not ultimately diagnosed. The natural history, evolution, management guidelines, and clinical course of this latter category of abruptio placentae appear sparsely presented or discussed in the medical literature, although these episodes represent the majority of encounters for most obstetricians.

In this nonacute category of abruptio placentae the diagnosis is often tenuous and rests on the subjective clinical impression that the bleeding results from premature, nonphysiologic separation of the placenta. The histologic examination is often nonconfirmatory after birth, particularly in the term laboring patient, after the complete and normal physiologic separation of the placenta has taken place. Histologic features are logically not present if the blood does not coagulate under the placenta or membranes but instead egresses through the cervix.

Without much descriptive literature of the nonacute abruptio placentae, numerous questions arise as to the appropriate management and ultimate outcome of this circumstance. Thus the particular objectives of this study were to examine such questions as whether there are hazards or benefits of tocolysis, what is the likelihood of a future extensive placental separation with acute distress or fetal loss with conservative management, and under what circumstances and for how long pregnancies may be safely maintained. In recent years ultrasound examinations have been reported in small

From the Department of Obstetrics and Gynecology, Evanston Hospital, Northwestern University School of Medicine.
Received for publication June 30, 1986; accepted August 14, 1986.
Reprint requests: John S. Sholl, M.D., 2650 Ridge Ave., Evanston, IL 60201.

Table I. Classification of abruptio placentae according to maternal and fetal signs

*Grade**	*Concealed hemorrhage*	*Uterine tenderness*	*Maternal hypotension*	*Coagulopathy*	*Fetal distress*
0	No	No	No	No	No
1	No	No	No	No	No
2	Yes	Yes	No	Rare	Yes
3	Yes	Yes	Yes	Often	Death

*Grade 0 is a retrospective diagnosis from inspection of the placenta. Grade 1 includes "marginal sinus" separation or other limited processes of abruptio placentae. Grades 2 and 3 imply significant hemorrhage requiring immediate therapy with the possibility of maternal complications.

series showing abruptio placentae findings.[12-14] Thus an additional issue is with what frequency ultrasound examination is capable of showing the abruptio placentae process and whether such clarification of the diagnosis offers any useful clinical parameters toward management of the patient.

The current report originates from the Evanston Hospital—a regional perinatal center serving the community of Evanston and surrounding north shore Chicago suburbs to the Wisconsin border. With use of computerized delivery data citing abruptio placentae as a delivery complication, cases were reviewed and clinical data tabulated to report on the broad spectrum of clinically diagnosed cases of abruptio placentae over 33 consecutive months.

Material and methods

Since July 1, 1983, delivery data at Evanston Hospital (labor delivery summary) have been computerized for the purpose of statistical analysis and review. With use of the clinical diagnosis of abruptio placentae as reported by the attending physician following delivery, patients were identified, their hospital charts reviewed, and clinical data abstracted into a study record encompassing July 1, 1983, to March 31, 1986. Several additional case records were obtained from the medical records department by search of the abruptio placentae ICD-9 code wherein the diagnosis of abruptio placentae was made at an antepartum hospitalization (without delivery), but without abruptio placentae being diagnosed at the subsequent hospitalization for delivery. One hundred thirty study records were accessed, limited to delivery between 20 to 42 weeks of estimated gestational age (best clinical estimate based on either last menses or ultrasound examinations), although several of the youngest gestational age cases (that is, <25 weeks) were managed as late abortions with nonviable severely premature fetuses. The abstracted data included gestational age at admission, presenting symptoms, gravity, parity, initial hemoglobin, hematocrit, platelet count, prothrombin time, partial thromboplastin time, low hemoglobin, low hematocrit, transfusions, tocolytic use, duration of use, clinical course, delivery indications, estimated gestational age at delivery, delivery type, fetal weight, Apgar scores, umbilical cord blood gases if obtained, and statements in the delivery or operative summary which reported findings such as marginally attached clots, excessive bleeding, definitive retroplacental clots. If ultrasound examination was done by the attending or house staff in the labor and delivery suite, the reported findings were entered for the record. Finally, the findings of ultrasound examinations performed by the obstetric ultrasonography laboratory during the presumptive course of the abruptio placentae were retrieved and entered on the study record. In anticipation of this report, and with the clinical suspicion of abruptio placentae, particular attention was directed to any abruptio placentae findings on ultrasound examination. The 130 case study records were thereafter entered in a computer file, enabling sorting and tabulation of the data. Admitting symptoms (one or more) were grouped with use of the admitting history terminology and physical descriptions: bleeding, spotting, chronic spotting (>4 weeks' duration), cramping, contractions, spontaneous rupture of membranes, hypertension or preeclampsia, back pain, or active labor. Delivery indications (one or more) were similarily categorized among the following from the clinical record: hemorrhage, active labor, cervical dilation, fetal distress (severe variable decelerations, late decelerations), fetal maturity, other. Surgical findings in the delivery or operation record were grouped as follows: old clots (judged to be well organized, fixed, or with friable quality), new clots, excessive bleeding, marginally attached clots, negative (no findings of abruptio placentae), vacant (no statements in the record), or simply abruptio placentae.

In reviewing the accumulated data regarding the objectives of the study, natural divisions became evident allowing placement of patients into three groups for analysis and discussion—those delivering severely prematurely at gestational week 20 to week 25 plus 3 days in whom fetal or neonatal death occurred without much effort or success in prolonging pregnancy (previable group); a middle group delivering between week 25 plus 6 days to week 36 plus 6 days of gestation (preterm

Table II. Demographic data of the three groups of the study population*—130 mothers and 135 infants

	Estimated gestational age at delivery		
	Term	*Preterm*	*Previable*
Age (yr)	29.5 ± 5.1	29.2 ± 5.4	29.4 ± 3.6
Range (yr)	18-43	16-45	24-34
Smoking habits			
Yes	3	15	1
No	30	25	4
Unknown	17	32	3
Race			
White	45	64	8
Black	2	6	0
Hispanic/Oriental	3	2	0
Primigravid	25 (50%)	18 (25%)	2 (25%)
1 term delivery with/without abortions	13 (26%)	20 (27.8%)	3 (37.5%)
2-5 term/preterm deliveries with/without abortions	8 (16%)	17 (23.6%)	1 (12.5%)
Only previous abortions or preterm delivery with neonatal loss	4 (8%)	17 (23.6%)	2 (25%)
Weight (gm)	1745†-4423	830-3218	250-780

*Term = Gestational week 37 to week 42 plus 1 day; preterm = week 25 plus 6 days to week 36 plus 6 days; previable = week 20 plus 2 days to week 25 plus 3 days.

†Intrauterine growth–retarded infant.

Table III. Symptoms at hospital admission

	Term		*Preterm*		*Previable*		*Total*	
	n	%	*n*	%	*n*	%	*n*	%
Bleeding (alone)	2	4	14	19.4	0		16	12.3
Bleeding with contractions/cramping	8	16	21	29.1	0		29	22.3
Bleeding with SROM	3	6	7	9.7	0		10	7.7
Contractions/active labor	11	22	4	5.6	0		15	11.5
SROM with/without cramping/labor	18	36	6	8.4	3*		24	18.5
Back pain with/without other symptoms	6	12	6	8.4	1*	12.5	13	10
Hypertension (preeclampsia)	1	2	4	5.6	1	12.5	6	4.5
Spotting/cramping	1	2	3	4.2	1	12.5	5	3.8
Chronic spotting with/without other symptoms	0		7	9.7	5*	62.5	12	9.2

SROM = Spontaneous rupture of membranes.

*In the previable group three patients sustained SROM with or after symptoms categorized above.

group); and last, the group delivering at a mature gestational age of >37 weeks for whom, in the face of suspected abruptio placentae or with the abruptio placentae process occurring during the course of active labor, maintenance of the pregnancy was not an issue and rarely undertaken. The middle group, those whose pregnancies were often able to be maintained with conservative management and/or tocolytic use, became the focal point of the report and of principal concern regarding the premature infant in the clinical management of nonacute abruptio placentae. The statistical methods used included χ^2 analysis with either the Yates correction or Fischer's exact test for small sample sizes and one application of the Kruskal-Wallis one-way analysis of variance.

Results

The mature group at delivery accounted for 50 mothers and 50 infants delivered at week 37 to week 42 plus 1 day of gestation. The demographic data are presented in Table II. The presenting symptoms are shown in Table III, which indicates a preponderance of patients presenting with spontaneous rupture of membranes and a distinct incidence of back pain occurring with any other symptoms. Within these term deliveries with abruptio placentae were three patients who had been previously admitted to the hospital with bleeding and a consequent clinical diagnosis of marginal sinus bleeding or limited abruptio placentae. These admissions were 79, 57, and 36 days before delivery. All three had been examined by ultrasound dur-

Table IV. Method of delivery according to estimated gestational age at delivery

	Term		*Preterm*		*Previable*		*Total*	
	n	%	*n*	%	*n*	%	*n*	%
Spontaneous	27	54	30	41.7	6	75	43	33.1
Low/mid forceps	7	14	1	1.4	0		8	6.2
Primary cesarean section	12	26	33	45.8	2	25	47	36.2
Repeat cesarean section	4	8	8	11.1	0		12	9.2
Cesarean section indications								
Hemorrhage	6		19		2		27	
Distress*	8		14		0		22	
Other	2†		8‡				10	

*Fetal distress as implied by abnormal fetal heart rate monitoring findings.
†Mature with visualized abruptio placentae, mature repeat cesarean section.
‡Nonvertex presentation, repeat cesarean section in labor, severe preeclampsia.

ing the previous admission, with negative findings for abruptio placentae. At delivery the placental examination was negative for evidence of the old abruptio placentae in two patients; however, old clots adherent to the placenta were evident in the 36-day patient. The remaining 47 patients did not have ultrasound examinations during the course of the labor and delivery, and 46 of the 47 were delivered within 0 to 1 calendar day of admission as expected. The mode of delivery is presented in Table IV.

Among the 16 cesarean sections, 14 were performed with a delivery indication of hemorrhage or fetal distress. The remaining two included one repeat cesarean section in labor and one primary case for which the diagnosis of abruptio placentae was made at the time of operation. The initial admitting diagnosis was abruptio placentae in nine cases. In seven of these delivery was expeditiously performed by cesarean section—one vaginally after measuring 7 cm at admission (3317 gm; Apgar scores, 8 and 9) and the other was the one with an abruptio placentae diagnosis 79 days earlier (3629 gm; Apgar scores, 8 and 9) with no abruptio placentae findings at delivery.

Neonatal outcomes of the term abruptio placentae patients were 48 living surviving infants with a weight range of 1745 to 4423 gm. These 48 infants had 5-minute Apgar scores of 8 to 10. Two infants were born with Apgar scores of 0 and 0, and both died. Baby C (3050 gm), who died on day 1 of life, was known to have a large diaphragmatic hernia antenatally with an 80% abruptio placentae occurring in the hospital the night before a planned repeat cesarean delivery. Baby W (3310 gm) was delivered by low forceps with rapid bright red bleeding occurring during the late second stage of labor. This severely asphyxiated infant lived 13 days. Operative report descriptions of the placenta and delivery course included 11 marginally attached clots, 30 cases of abruptio placentae, 17 instances of excessive bleeding, seven new clots, two old clots, and four negative or vacant statements.

Review of the predelivery and postdelivery hemoglobin/hematocrit values and platelet and clotting assays in the mature group showed nominal blood loss in the great majority with many patients lacking postdelivery values. Three patients received 1 to 2 U transfusions. Patient C, whose infant was described above and who had the massive abruptio placentae in hospital, received 4 U of packed red blood cells. Additionally, this was the only patient among 15 assayed who had clotting dysfunction. Platelet counts were obtained in 20 of the 50 term abruptio placentae patients and were $<150{,}000/mm^3$ in only four instances. The average hemoglobin drop of these four was 2.6 gm/100 ml and, given the wide variation, was not statistically different from the mean 2.1 gm/100 ml drop in hemoglobin of 12 other patients (with available data) whose platelet counts were $>150{,}000/mm^3$.

At the previable gestational ages of 20 weeks to 25 weeks plus 3 days, eight cases were segregated based on the gestational age and outcome similarities. Their demographic data are also presented in Table I. Five patients reported chronic spotting (at least 4 weeks) as a presenting symptom with cramping or contractions at admission. Two with this chronic spotting and a third patient who complained of back pain, sustained spontaneous rupture of membranes leading to admission. The seventh patient presented with severe preeclampsia at gestational week 23 plus 6 days. The eighth patient in the group presented with spotting and active preterm labor at 10 cm dilation. The initial diagnosis was abruptio placentae in four of the eight, with the one preeclampsia case and three additional cases diagnosed as having preterm labor completing the group. The three patients in active preterm labor were delivered vaginally, as were three others—two with successful prostaglandin evacuations. Two patients underwent

Table V. One- and 5-minute Apgar scores in the preterm group according to delivery type

	No. of infants delivered by cesarean section grouped by Apgar score (n = 45)				*No. of infants delivered vaginally grouped by Apgar score (n = 31)*			
	7-10	*4-6*	*1-3*	*0*	*7-10*	*4-5*	*1-3*	*0*
At 1 minute	19	15	8	3	18	10	2	1*
At 5 minutes	39	3	1	2†	28	2	0	1*

*Known intrauterine death before delivery.

†One known intrauterine death secondary to acute hemorrhage, in transverse lie, and a previous intrauterine death of a 500 gm growth-retarded twin.

Table VI. Comparison of delivery method and indications in preterm patients with visible clots, no findings of abruptio placentae, other findings, and not scanned

	n	*Primary cesarean section*	*Repeat cesarean section*	*Vaginal delivery*	*Indications for cesarean section*		
					Hemorrhage	*Distress*	*Other*
Visible clot	12	8	0	4	6	1	1*
No abruptio placentae	33	11	3	19	6	5	3†
Other findings‡	3	1	1	1	0	2	0
Not scanned	24	13	4	7	8	6	3§
Total preterm	72	33	8	31	20	14	7

*Fetal maturity with visible retromembranous clot.

†Active labor, repeat cesarean section; severe preeclampsia; breech presentation.

‡Oblique septum, intrauterine fetal death witnessed, rounded placenta.

§A pair of 850 and 830 gm twins; severe preeclampsia; transverse lie.

hysterotomy because of hemorrhage. Six of the group had had ultrasound examinations, and in two, extensive retromembraneous dissections were clearly visible, whereas in four there was no finding of abruptio placentae. Although there were only two positive scan findings of abruptio placentae, four of the six patients who were scanned did indeed have old adherent clots found at delivery.

All these previable neonates died intrapartum or in the immediate neonatal period as expected. Only two pregnancies were maintained >2 days from admission to delivery, which included one case with a sonographically visible extensive retromembraneous dissection seen at 17 weeks' gestation with eventual hysterotomy at 20 weeks plus 2 days for hemorrhage and another case with spontaneous rupture of membranes at 20 weeks, subsequent chronic spotting, and then hemorrhage at 24 weeks plus 3 days, also requiring hysterotomy. Among these patients only the one with severe preeclampsia had thrombocytopenia (140,000/mm^3), and none had elevated prothrombin time or partial thromboplastin time values. None of these patients were given tocolytics.

Seventy-two patients who were delivered between 25 weeks plus 6 days and 36 weeks plus 6 days made up the preterm group of patients. Demographic data are presented in Table II. In comparison to the term pregnancies, there was no difference in age but a statistically significant increase in the number of known cigarette smokers. A χ^2 analysis of smoking/nonsmoking or unknown and preterm/term abruptio placentae in a two-by-two table yielded a value of 5.162 ($p < 0.025$). There was also a significantly increased proportion of preterm patients reporting a poor obstetric history, that is, 17, or 23.6%, had had only abortions or preterm deliveries without surviving infants ($\chi^2 = 10.279$ at 3 df ($p < 0.025$).

Presenting symptoms are shown in Table III. A review of the composite data in this table underscores the wide range of presenting symptoms in abruptio placentae and the variation of those symptoms according to gestational age groups. Of note is the 10% incidence of back pain, which thus appears to be a quite specific signal for abruptio placentae. The total of the "chronic spotting," "back pain" and the totaled "bleeding" rows equals 85 patients, which implies that the remaining 45 of 130 or about one third of patients presented without symptoms that would ordinarily imply that an abruptio placentae was occurring or about to occur. This proportion was verified by tabulation of the "initial diagnosis" entries derived from the initial history and physical, wherein 48 of the 72 preterm patients (66.7%) were diagnosed to have abruptio placentae or marginal sinus bleeding at admission. Only four patients in this preterm group (and therefore six in the entire series) had blood pressure values ≥130/90 mm Hg. The completed

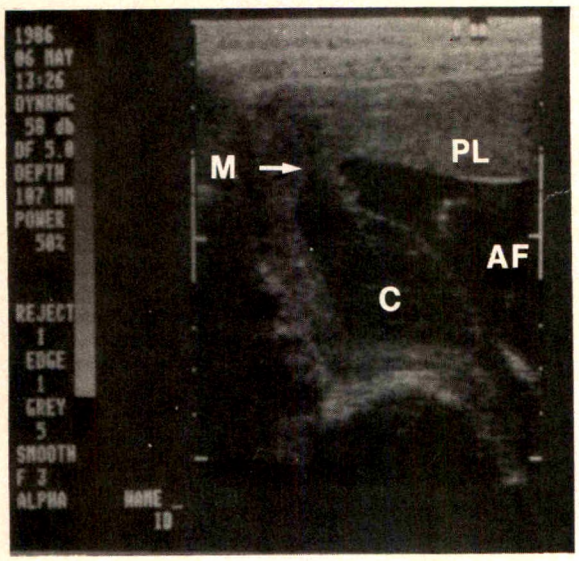


Fig. 1. This transverse linear-array ultrasound photograph shows an anterior placenta *(PL)*, normal amniotic fluid *(AF)*, and an extensive retromembranous blood clot *(C)*, originating from the placental margin *(M)*.

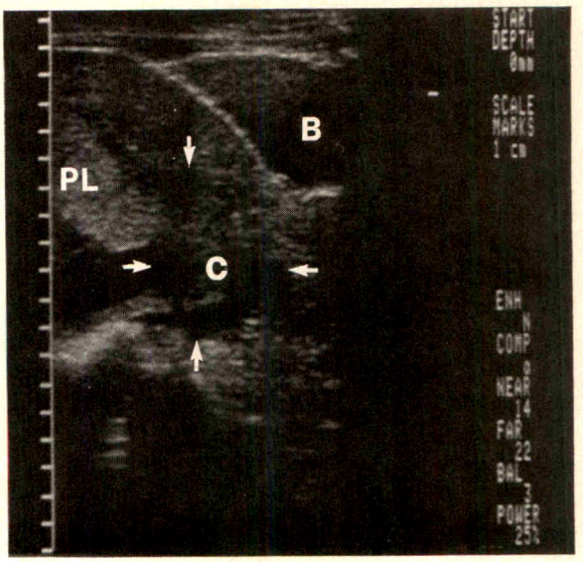


Fig. 2. This longitudinal linear-array ultrasound photograph shows the bladder *(B)*, a low-lying placenta *(PL)*, and the fluid and solid echoes of an abruption clot *(C)*, outlined by *arrows*. The placental edge is elevated by some dissection of the blood underneath.

Table IV also shows the delivery methods for the three gestational age groups. In the preterm group 57% of the gravid women were delivered by cesarean section and, of these, 80.5% on an emergency basis for either hemorrhage or fetal distress. Twenty-three patients received transfusions of 1 to 4 U of blood with a total of 48 U transfused. Seventeen of these patients receiving transfusions were delivered by cesarean section compared to six with vaginal deliveries. Platelet counts were $<150{,}000/mm^3$ in six patients, three of whom were hypertensive. The mean hemoglobin drop (admitting value to nadir value) was 3.78 ± 1.36 gm/100 ml for these six compared with 1.88 ± 1.27 gm/100 ml for 55 others with available data.

A comparison of the immediate neonatal condition of the infants delivered vaginally compared to those by cesarean section in the preterm group is given in Table V.

Appropriately timed ultrasound examinations were performed in 48 of the 72 preterm patients. Of the 24 patients not scanned, 13 were delivered the day of admission and 22 within 5 days of admission. The delivery method for 17 of the 22 was cesarean section with assorted indications—two with active labor (a 1420 gm fetus in transverse lie and 830 and 850 gm twins); eight for hemorrhages; six for fetal distress; one breech with preeclampsia. There were six vaginal deliveries—five with active labor/cervical dilation and one with preeclampsia. The two patients who were delivered without scans and with hospital stays >5 days both had the abruptio placentae occurring as a late event. One patient had had spontaneous rupture of membranes 44 days earlier, with bleeding commencing the day before cesarean delivery for hemorrhage, and the other patient was hospitalized for hypertension and fetal growth retardation when hemorrhage and fetal distress began on the twelfth hospital day.

In the 48 scanned patients, 33 examinations showed no evidence of abruptio placentae. Six retroplacental clots and six retromembranous clots were visualized, confirming the abruptio placentae diagnosis. Examples of the ultrasound findings are shown in Figs. 1 and 2. One rounded placental contour was noted, which is also a diagnostic feature (but not conclusive) of abruptio placentae.[11] At delivery old clots were reported in 11 of the 33 negative examinations while the remaining 22 patients had delivery record reports of "excessive bleeding," "new clots," "marginal (attached) clots," or the nondescriptive statement of "abruption." Old clots were evident in all 12 patients with sonographically visualized retroplacental or retromembranous clot formation. With use on a hypothetical basis, that the presence of old clots at delivery implies that the diagnosis could theoretically have been made by ultrasound, the sensitivity is derived as 12 of 23 (52.2%). Overall, however, ultrasound examination confirmed the diagnosis in only the 12 of these 48 scanned patients (25%). In a comparison of cases with visualized clots to those with negative findings of abruptio placentae, there were no differences in mode of delivery (Yates' corrected $\chi^2 = 1.21$, $p = 0.27$, in a two-by-two table of visible/negative compared to cesarean section or vaginal de-

Table VII. Preterm patients categorized by presenting symptoms, tocolytic use, delay to delivery, delivery indications, and delivery route

Group	Delay to delivery (days)	n	Delivery indications			
			Active labor/ cervical dilation	Hemorrhage	Fetal distress	Other
Group A*						
	0-3	28	12	9	5	2†
	4-10	11	4	6	1	0
	11-21	4	3	0	1	0
	>22	7	1	5	1	0
Total		50	20	20	8	2
Group B‡						
	0-3	8	1	3	2	2
	4-10	3	1	1	1	0
	11-21	7	3	0	3	1§
	>22	4	3	1	0	0
Total		22	8	5	6	3

*Cramping, contractions, SROM, active labor with/without bleeding, spotting, preeclampsia, or back pain.
†One intrauterine fetal death and one case of preeclampsia delivered vaginally.
‡Bleeding, back pain, and preeclampsia without cramps, contractions, SROM, or active labor.
§One primary cesarean section with visualized abruptio placentae and demonstrated lung maturity.

Table VIII. Preterm patients grouped according to use of tocolysis,* delay to delivery, delivery indications, route of delivery, and use of steroids

Delay to delivery (days)	n	Delivery indications				Delivery route			Timely use of steroids†
		Active labor/ cervical dilation	Hemorrhage	Fetal distress	Other	Primary cesarean section	Repeat cesarean section	Vaginal	
No tocolysis									
0-3	23	6	8	5	4‡	13	2	8	0
4-10	3	0	1	2	0	3	0	0	1
11-21	5	3	0	1	1§	1	1	3	1
>22	2	2	0	0	0	0	0	2	0
Total	33	11	9	8	4	17	3	13	2
Tocolysis									
0-3	13	7	4	2	0	6	2	5	6
4-10	11	5	6	0	0	4	1	6	4
11-21	6	2	0	3	1*	3	1	2	3
>22	9	2	6	1	0	3	1	5	2
Total	39	16	16	6	1	16	5	18	15

*Tocolysis used: 10, magnesium sulfate, intravenous infusion; 13, ritodrine by intravenous infusion or orally; 16, sequential magnesium sulfate, intravenous infusion and ritodrine intravenous infusion or orally.
†Timely use of steroids implies delivery occurred 2 to 7 days after start of dexamethasone therapy, 5 mg every 12 hours, by intramuscular injections for four doses.
‡Two preeclampsia, one amnionitis, one intrauterine fetal death.
§Demonstrated lung maturity with visualized clot on sonogram.

livery) and no differences in the indications for cesarean delivery (both Yates' correction and Fischer's exact test yield p = 1.00 in a two-by-two table of visible/negative versus hemorrhage or fetal distress/other delivery indication).

The interval between admission and delivery in the 12 patients with positive sonographic examinations for abruptio placentae varied from 0 to 85 days with a mean ± SD of 13.4 ± 23.6 days. With no evidence of abruptio placentae the interval between admission and delivery ranged from 0 to 45 days with a mean ± SD of 13.1 ± 13.4 days. If the unusual case with 85 days from initial abruptio placentae to delivery interval is dropped, the mean ± SD with a visualized clot was 6.9 ± 7.5 days (range, 0 to 18 days). Further comparison of the sonographically visible cases compared to

Delivery route		
Primary cesarean section	*Repeat cesarean section*	*Vaginal*
12	4	12
5	1	5
0	1	3
2	1	4
19	7	24
7	0	1
2	0	1
4	1	2
1	0	3
14	1	7

those with no evidence of abruptio placentae is shown in Table VI.

In the patients with positive findings of abruptio placentae there were two stillbirths (one before admission and the other within 30 minutes of admission) and three neonatal deaths (480 gm on day 0, 1701 gm at 24 days of age, and 1925 gm on day 12 of life). In the group with no finding of abruptio placentae there were five neonatal deaths: twins of 960 and 850 gm both on day 13 of life, a 1050 gm twin on day 23, 1200 gm on day 11, and a 1315 gm infant on day 0. The 1050 gm twin and the 1315 gm infant both had pulmonary hypoplasia from long-standing oligohydramnios.

In an examination of the use and nonuse of tocolysis in the preterm group, the 72 cases were first divided into subsets according to indications for tocolysis and then reviewed as to success, failure, delivery indications, and finally neonatal outcome. This classification of patients by presenting symptoms is shown in Table VII.

With symptoms of uterine activity at admission, 28 of 50 (56%) were delivered within 3 days compared to eight of 22 (36.4%) if these symptoms were not present. The deliveries at 4 to 10 days (22% versus 13.6%), 11 to 21 days (8% versus 31.8%), and >22 days (14% versus 18.2%) demonstrate the trend of earlier delivery with uterine activity present. With symptoms implying uterine activity (cramping, contractions, spontaneous rupture of membranes, or active labor) 30 of the 50 patients received tocolytic therapy. Those not receiving tocolytic therapy from admission date to delivery remained undelivered from 0 to 44 days, but 15 of the 20 were delivered within 2 days for the indications evident in Table VII, namely, advanced cervical dilation or active labor, fetal distress (which might include hemorrhage), hemorrhage (without fetal distress), or preeclampsia. The five delayed deliveries were at 4, 9, 17, 18, and 44 days, respectively, and included one patient with long-term spontaneous rupture of membranes who had abruptio placentae as a late event in the course of her hospitalization and two others with cramping, spotting, or contractions, who were delivered 4 and 18 days later. Two other patients admitted 17 and 44 days before delivery with spotting and cramping were diagnosed as having limited abruptio placentae—one having a "negative" report for findings of abruptio placentae at delivery and the other with old clots evident at delivery. Insofar as the route of delivery in the uterine activity group is concerned, there was no discernible change as a function of delay to delivery, with a comparable number of cesarean versus vaginal deliveries at each delay interval. Worthy of note is the finding that the delivery after prolonged delay, that is, >10 days, often was prompted by evolution to an "acute" abruptio placentae problem with hemorrhage or fetal distress in seven of 11 (63.6%). In this preterm group with uterine activity symptoms, overall there were 26 cesarean deliveries versus 24 vaginal births.

In the 22 patients with bleeding, back pain, or hypertension as presenting symptoms exclusive of spontaneous rupture of membranes, cramps, contractions, or active labor, 13 (59%) did not receive tocolytic therapy. These 13 patients were delivered within 0 to 34 days from initial hospital admission, eight of them being delivered within 2 days. The remaining five include a patient with a large uterine septum spending 9 days in the hospital before cesarean section delivery for hemorrhage; a patient with twins, bleeding, subsequent advanced cervical dilation, and cesarean section delivery on day 12; the patient with abruptio placentae on day 12 of hospitalization with preeclampsia and an intrauterine growth–retarded fetus; a patient admitted for bleeding 15 days before spontaneous delivery with a marginal abruptio placentae evident at the 36-week delivery; and finally one patient admitted with bleeding 34 days before delivery who had "negative" delivery findings after spontaneous delivery at week 36 plus 6 days. Nine patients admitted with bleeding, back pain, or hypertension symptoms subsequently received tocolytic therapy with admittance-to-delivery intervals of 4 to 85 days—4, 6, 12, 15, 18, 21, 22, 29, and 85, respectively.

In contrast to the uterine activity preterm subset, there were twice as many total cesarean section deliveries compared to the vaginal (15 versus seven compared to the 26 versus 24) but in this nonuterine activity subset there also was not a discernible trend over time with delay to delivery and delivery route. The eight patients who were delivered within 3 days account for

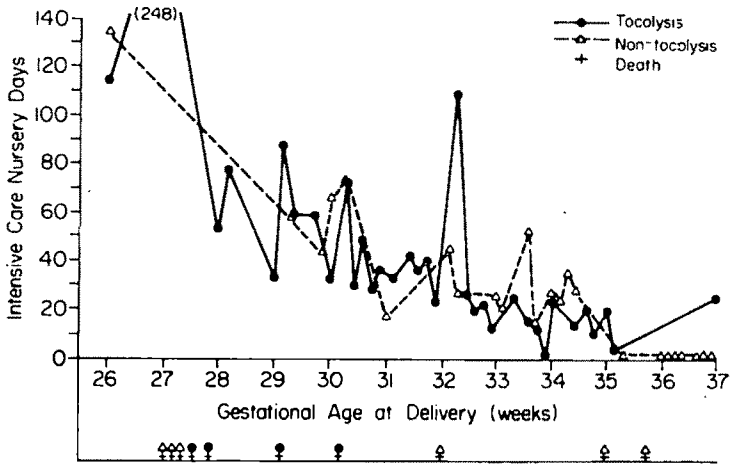


Fig. 3. Length of stay in the intensive care nursery *(ICN)* by days is plotted against gestational age at delivery for infants whose mothers received tocolysis *(solid line)* and those who received no tocolysis *(dashed line)*. The plot show no statistical difference affirming that gestational age is the principal determinant of intensive care unit stay. Fetal or neonatal deaths are shown at the bottom, according to gestational age at birth.

seven of the 14 primary cesarean sections done. Thus once beyond the 3-day delay, there was a rather equal likelihood of vaginal compared to the cesarean delivery at each delay interval and similarly a good chance (six of 14, or 42.9%) that the delayed delivery would be prompted by an emergency situation such as hemorrhage or fetal distress. Four patients in the preterm group presenting without symptoms of uterine activity had admission-to-delivery delay >3 weeks (22, 29, 34, and 85 days). Old clots were evident at delivery in the first three of these patients whereas the delivery examination was "negative" in the patient admitted 85 days before delivery with bleeding diagnosed as a "limited or marginal abruption" not visible on sonogram. In the totaled 72 preterm patients there were 41 cesarean sections (57%) compared to the 31 vaginal (43%) deliveries and 39 (54%) emergency indications of fetal distress and/or hemorrhage.

In Table VIII the 72 preterm patients are segregated according to use or nonuse of tocolysis. Of the 33 patients not receiving tocolysis 10 (30.3%) had delay to delivery of >3 days compared to 26 of 39 (66.7%) patients with use of tocolytics. If the 0- to 3-day delay deliveries (that is, the urgent or emergency cases with advanced preterm labor, significant hemorrhage, or fetal distress) are not considered, the 26 remaining patients receiving tocolysis gained an average of 19.9 ± 18.3 additional days (mean ± 1 SD with SE of the mean = 3.6 days) compared to 17.5 ± 12.3 additional days (mean ± 1 SD with SE of mean = 3.9 days) for the 10 remaining patients not receiving tocolysis. On a statistical basis the gain in days is not significant (Kruskal-Wallis one-way analysis of variance, $p = 0.96$), but there are obvious selection biases in the tocolysis versus nontocolysis divisions—those patients receiving tocolysis were more often those with preterm labor or uterine activity or prodromal symptoms (cramping, spontaneous rupture of membranes, contractions), and those who were delivered within hours or a few days of admission because of hemorrhage were excluded.

When the cases with use of tocolysis are compared to those without its use, there is no discernible change regarding route of delivery (60.6% versus 53.8% by cesarean section). Similarly there is no discernible relationship by tocolysis/no tocolysis divisions nor as a function of delay to delivery, regarding acute indications for delivery, with 17 of 33 (51.5%) and 22 of 39 (56.4%) having fetal distress and/or hemorrhage prompting delivery in the two subsets, respectively. Some bias may be eliminated by considering only those patients delivering after 3 days in the tocolysis/no tocolysis divisions. Again there is no effect on mode of delivery (Fischer's exact test, $p = 1.00$, for two-by-two table of tocolysis/no tocolysis beyond 3 days from admission vs. cesarean section/vaginal delivery) nor on the delivery indications (Fischer's exact test, $p = 0.28$, for two-by-two table of tocolysis/no tocolysis beyond 3 days from admission vs. hemorrhage or fetal distress/other delivery indication). Despite an increased timely use of steroids in the tocolyzed patients, there was no evident reduction in days in the intensive care nursery for the neonates. This is shown in Fig. 3 with an overlay of fetuses with tocolysis compared to the fetuses with no tocolysis and length of intensive care nursery stay in days, as a function of estimated gestational age at delivery.

For the total 72 preterm pregnancies the probability of not being delivered as a function of days from ad-

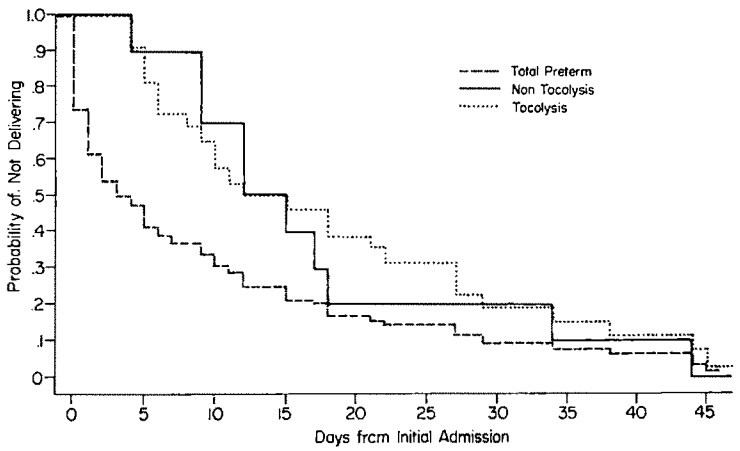


Fig. 4. The plots show the probability curves of not being delivered as a function of days from initial admission for the preterm group. The *dashed line* shows all 72 patients in this group. The *dotted* and *solid* lines show only patients being delivered after 3 days from admission with a comparison of tocolysis (n = 26) and nontocolysis (n = 10) suubsets.

mission is shown in Fig. 4. Twenty-six percent were delivered the day of admission and 50% within 3 days of admission. If those patients who were delivered within 3 days are excluded a more legitimate comparison of the effect of the use of tocolysis compared to the nonuse of tocolysis can be made. Also shown in Fig. 4 is a comparison of the probability of not being delivered as a function of days beyond 3 days for the 10 and 26 patients remaining undelivered at the third day in the nontocolysis and tocolysis divisions respectively. Finally, Fig. 5 shows the probability of delivery by 3, 10, and 21 days as a function of gestational age at presentation for the entire 130 patients in the series.

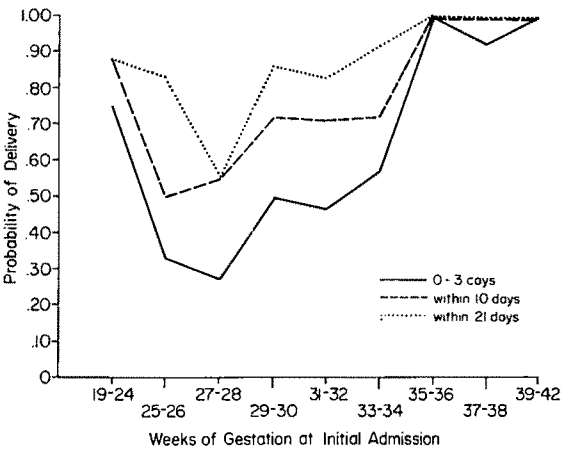


Fig. 5. This plot shows the probability of delivery within 3, 10, and 21 days of admission for the entire study population (n = 130) as a function of gestational age (in 2-week blocks) at initial hospital admission.

Comment

This study examined retrospectively the broad spectrum of bleeding episodes in pregnancy clinically diagnosed as abruptio placentae from 20 to 42 weeks at a level III perinatal center hospital serving principally a middle- to upper-class population. The major emphasis of the report is to contrast the wide individual variations in acuity of abruptio placentae and its course in a day-to-day practice with the more frequent literature and textbook focus on the severe cases of abruptio placentae, which note maternal risk factors, rapid progression to fetal death or distress, and maternal coagulopathy or hypovolemic shock complications. Similarly these sources highlight the urgency mandated to reduce perinatal and maternal morbidity and mortality. In the classic study of Pritchard et al.[7] of severe abruption causing fetal death in utero, 45% of the gravid women were hypertensive. Abdella et al.[9] similarly reported a 26.8% incidence of hypertensive disorders. Within the current study however, only six of the 130 patients (4.5%) presented with hypertension as a notable factor, and hypertension was particularly rare with abruptio placentae occurring at term. Known cigarette smoking was more frequent in preterm versus term abruptio placentae although this finding is somewhat suspect given the large proportion of "unknown" smoking habits. A difference in parity was similarly evident in term versus preterm abruptio placentae, showing that greater parity and particularly only abortions or prior preterm perinatal losses were more frequent in the preterm.

Knab,[5] in reviewing the results of several other authors,[1-5, 11] emphasized that cesarean delivery was without question associated with a decreased perinatal mortality compared to vaginal delivery. In a tabulation of seven reported series, the total perinatal mortality for vaginal delivery was 31.5% compared to 16.7% for ce-

sarean delivery. His recommendation for urgent cesarean delivery was qualified to pertain to moderate and severe cases of abruptio placentae at ≥31 weeks (1500 gm) but included the statement that with mild abruptio placentae also "long delays do not seem to be in the best interest of the fetus." In the present study the overall perinatal mortality was much lower, and logically so, since the broad range of mild to severe abruptio placentae was reviewed. The perinatal losses included the two term infants discussed above, the eight previable infants, and 13 preterm infants for a total of 23 (17%). With corrections (11 infants of <800 gm, a stillborn infant with nonimmune hydrops fetalis and omphalocele, and three infants with pulmonary hypoplasia), the perinatal mortality was eight of 130 (6.2%) with four delivered by cesarean section and three vaginally.

The low perinatal mortality rate in part is derived from inclusion of mild or grade 0 cases of abruptio placentae but also affirms the appropriateness of prompt delivery, especially as was carried out in the patients presenting at term. The results in the term group imply that fetal heart rate monitoring (which at our institution commences immediately on admission and is nearly continuous thereafter) is sufficient to identify those fetuses with fetal distress, so that cesarean sections can be performed in advance of a fetal loss. In the infants delivered at a previable gestational age, the issue of delay compared to the aggressive effort to maintain the pregnancy is moot, since the assignment to this group is self-fulfilling regarding perinatal death. In the preterm delivery group, however, there were 10 patients who were initially admitted in weeks 25 to 26 of pregnancy, who then maintained the chronic abruptio placentae process passing into the preterm group. For the preterm delivery group, clinical managment at admission again included immediate fetal heart rate monitoring with assessment of maternal status. On the basis of clinical course, 36 were delivered within 3 days of admission, but the remaining 36 were maintained with a broad range of days and weeks beyond. A few of this group were discharged after fulfilling the prerequisites of not bleeding for 7 days, not having any fetal heart rate monitoring abnormalities, and not having ongoing uterine cramping or contractions. Under these guidelines, most patients did not qualify for discharge and remained hospitalized under management protocols that included daily heart rate monitoring and liberal use of tocolysis to stop uterine activity. Although, as shown in Table VIII, there was frequent progression to rapid hemorrhage and or fetal distress necessitating delivery (with an overall 56.9.% cesarean section rate), there were no antepartum fetal deaths in this group and only rare low Apgar scores with either vaginal or cesarean delivery (Table V). This further implies that inpatient surveillance for onset of hemorrhage, frequent fetal heart rate monitoring, and immediate access to cesarean delivery, often allowed significant advancement in gestational age without perinatal losses from sudden catastrophic extension of the abruptio placentae. Patience and effort to prolong the pregnancy, combined with this close surveillance, appear to have been very beneficial, albeit on an individual and retrospective assessment—not based on a comparison of immediate delivery to conservative management prospectively.

As a retrospective review in contrast to a prospective randomized treatment protocol, the usefulness of tocolysis with abruptio placentae is not established because the patients receiving tocolysis compared to those not receiving tocolysis differed by presenting symptoms, clinical course, the urgency of delivery indications, and by differences in the various physicians' practice habits. However, if the selection biases regarding use of tocolysis compared to no tocolysis in the preterm group are viewed in some perspective, there appears to be some success in prolonging pregnancy by use of tocolysis. The curves in Fig. 4, showing the probability of not delivering with/without tocolysis if the pregnancy is maintained beyond 3 days from initial admission, at least suggest that the pregnancies with abruptio placentae receiving tocolysis for the usual indications of cramping or contractions can be maintained a number of days commensurate to those of pregnancies in which uterine activity was presumptively not occurring. On the positive side, the results in Table VIII do not support a notion that tocolysis increases the hazard of fetal distress or hemorrhage or that it increases the likelihood of cesarean delivery.

Preferentially, magnesium sulfate was used initially for tocolysis so as not to distort maternal and fetal heart rates as an assay of maternal blood loss or fetal condition. Eventual use of oral ritodrine was a minor goal of treatment, and many patients were maintained chronically on intravenous infusion therapy of either magnesium sulfate or ritodrine, since hospital discharge was infrequent. The study did not encompass a comparison of magnesium sulfate with ritodrine for tocolysis, and when either drug was effective, it was often maintained without changes.

The use of ultrasound to confirm the diagnosis of abruptio placentae shows limited success in the study, and its principal function was to refute the presence of a placenta previa. As would be expected, it played an insignificant, if nonexistent, role in the term group, in which maternal and fetal condition were of principal importance in clinical management. Obviously, in grade 2 abruptio placentae with fetal distress present, ultrasound visualization of the retained clot might be possible and clinically satisfying but is of no consequence in the decision to deliver the patient on an emergency

basis. In the preterm group, visualization of the clot had no impact on the likelihood of cesarean compared to vaginal delivery or on the likelihood of having hemorrhage or fetal distress rather than another indication for cesarean delivery. In any event, even with visualization of a clot, there was a fair chance that the pregnancy could continue for some time, with an absolutely certain diagnosis of abruptio placentae. The ultrasound visualization of abruptio placentae does, however, confound its grading by the Page system (Table I), in that concealed hemorrhage may be present in grades 0 or I.

In summary, this study has shown that there is a wide range of acuity with abruptio placentae, such that in many instances pregnancy can be successfully maintained without evident hazard to mother or fetus. The use of tocolysis with abruptio placentae was found to have no documented deleterious effects and generally was felt to be helpful in prolonging pregnancies with a chronic process of abruptio placentae occurring. Cigarette smoking and previous poor obstetric history were found to be associated with preterm abruptio placentae. Hypertension and thrombocytopenia and clotting dysfunction were rare but seemingly related. Ultrasound visualization of a clot was successful in 25% of cases, with a hypothetical sensitivity of 52%. Finally, inpatient management with frequent fetal heart rate monitoring is credited for allowing advancement in gestational age and reduced perinatal mortality.

REFERENCES

1. Hibbard BM, Jeffcoate TNA: Abruptio placentae. Obstet Gynecol 1966;27:155.
2. Page EW, King EB, Merrill JA. Abruptio placentae: dangers of delay in delivery. Obstet Gynecol 1954;3:385.
3. Golditch IM, Boyce NE. Management of abruptio placentae. JAMA 1970;212:288.
4. Lunan CB. The management of abruptio placentae. J Obstet Gynaecol Br Commonw 1973;80:120.
5. Knab DR. Abruptio placentae: an assessment of the time and method of delivery. Obstet Gynecol 1978;52:625.
6. Hurd WW, Miodovnik M, Hertzberg V, Lavin JP. Selective management of abruptio placentae: a prospective study. Obstet Gynecol 1983;61:467.
7. Pritchard J, Mason R, Corley M, Pritchard S. Genesis of severe placental abruption. AM J OBSTET GYNECOL 1970; 108:22.
8. Naeye RL, Harkness WL, Utts J. Abruptio placentae and perinatal death: a prospective study. AM J OBSTET GYNECOL 1977;128:740.
9. Abdella TN, Sibae BM, Hays JM, Anderson GD. Relationship of hypertensive disease to abruptio placentae. Obstet Gynecol 1984;63:365.
10. Sher G. A rational basis for the management of abruptio placentae. J Reprod Med 1978;21:123.
11. Blair RG. Abruption of the placenta. J Obstet Gynecol Br Commonw 1973;80:242.
12. Spirt BA, Kagen EH, Rozanski RM. Abruptio placentae: sonographic and pathologic correlation. AJR 1979;133: 877.
13. Jaffe MH, Schoen WC, Silver TM, Bowerman RA, Stuck KJ. Sonography of abruptio placentae. AJR 1981;137: 1049.
14. Rivera-Alsina ME, Saldano LR, Maklad N, Korp S. The use of ultrasound in the expectant management of abruptio placentae. AM J OBSTET GYNECOL 1983;146:924.

Randomized management of the second nonvertex twin: Vaginal delivery or cesarean section

Jaron Rabinovici, M.D., Gad Barkai, M.D., Brian Reichman, M.B., Ch.B., David M. Serr, M.B., Ch.B., and Shlomo Mashiach, M.D.

Tel-Hashomer and Tel Aviv, Israel

Sixty twin deliveries after the thirty-fifth gestational week with vertex-breech and vertex-transverse presentations were managed according to a randomization protocol. Thirty-three parturient women (21 vertex-breech and 12 vertex-transverse presentations) were allocated for vaginal delivery and 27 for cesarean section (18 vertex-breech and nine vertex-transverse). Six pairs of twins in the vaginal delivery group were delivered in a different mode than requested by the protocol (two women underwent cesarean section; in four cases the second twin spontaneously changed to vertex presentation). There were no significant differences between 1- and 5-minute Apgar scores and incidence of neonatal morbidity between the second-born twins in both study groups. Firstborn twins had higher 1-minute Apgar scores than the second-born infants irrespective of route of delivery ($p < 0.05$). No case of birth trauma or neonatal death was recorded. Maternal febrile morbidity was significantly higher in the cesarean section group than in the vaginal delivery group (40.7% versus 11.1%, $p < 0.05$). These results suggest that in twins with vertex-breech or vertex-transverse presentations after the thirty-fifth week of gestational age the neonatal outcome of the second twin was not significantly influenced by the route of delivery. (Am J Obstet Gynecol 1987;156:52-6.)

Key words: Twin gestation, delivery, cesarean section

The optimal route of delivery for a twin pregnancy with vertex-breech or vertex-transverse presentations has remained a subject of controversy. Cesarean section has been advocated by some clinicians as the appropriate mode of intrapartum management, since vaginal delivery of the second nonvertex twin was associated with adverse perinatal outcome.[1-7] During the last years several studies have suggested that the second nonvertex twin weighing >1500 gm can be delivered vaginally with as much safety as by cesarean section.[8-10] These contradictory results have been partly attributed to the retrospective design of all previous studies.[9,11]

This report presents our experience with the randomized intrapartum management of vertex-breech or vertex-transverse twin gestations. The protocol was instituted in order to evaluate the influence of the route of delivery on neonatal and maternal outcome.

Material and methods

Selection and preparation of patients. The study period ranged from March 1, 1983, to September 30, 1985. The staff of the Department of Obstetrics and Gynecology at the Chaim Sheba Medical Center was instructed to consider every patient with a twin pregnancy presenting to the labor ward for participation in this study. Fetal confinements were assessed by a plain abdominal x-ray film and by an ultrasound examination that included estimation of fetal weight, amniotic fluid volume, localization of the placenta and umbilical cord, and exclusion of gross fetal anomalies.

The following inclusion criteria were requested: twin pregnancy with spontaneous or induced labor and with both twins alive, the first fetus in vertex presentation and the second twin in breech presentation or in transverse lie, estimated gestational age between 35 and 42 weeks, presumably normal fetal anatomy, no signs suggestive of acute placental insufficiency or abruptio placentae, normal amniotic fluid volume, normal fetal heart rate testing, no maternal or obstetric indications for a specific route of delivery (such as cephalopelvic disproportion, previous uterine surgery, or uterine anomalies that contraindicated vaginal delivery), and cervix <7 cm dilated. Cervical dilatation was included as a criterion in order to allow sufficient time to prepare for a cesarean section.

The route of delivery was determined after the fulfillment of the above criteria according to a protocol that was changed randomly by a noninvolved person without prior notice on a time basis. If the parturient patient was found suitable to participate, the senior

From the Departments of Obstetrics and Gynecology and Neonatology, Chaim Sheba Medical Center, Tel-Hashomer, and the Sackler School of Medicine, Tel Aviv University.

Received for publication March 6, 1986; revised June 30, 1986; accepted July 12, 1986.

Reprint requests: Jaron Rabinovici, M.D., Department of Obstetrics and Gynecology, Chaim Sheba Medical Center, 52 621 Tel-Hashomer, Israel.

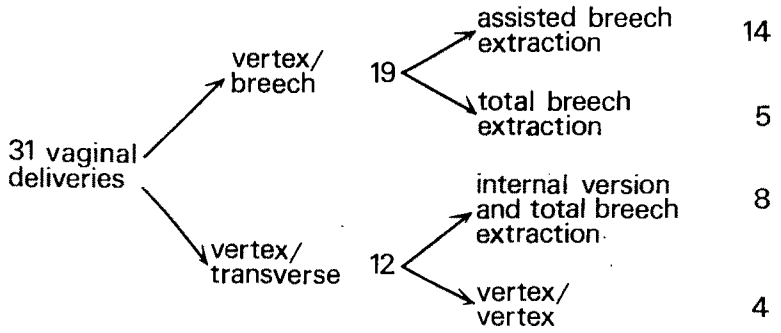


Fig. 1. Presentations and mode of delivery of the 31 pairs of nonvertex twins delivered vaginally according to randomized allocation. (Note that two women allocated to the vaginal group but delivered by cesarean section were not included.)

obstetrician inquired about the proposed mode of delivery and labor was managed accordingly. The protocol of this study was approved by the local ethical comittee of the Chaim Sheba Medical Center.

In order to achieve uniformity in the management of labor the following guidelines were established: In the cesarean section group the delivery was undertaken through a lower-segment transverse uterine incision. The preferred mode of anesthesia was epidural analgesia, but this depended ultimately on the anesthesiologist's decision. In the group delivered vaginally the progress of labor was evaluated by Friedman curve. Fetal monitoring consisted of continuous electronic recording by external ultrasonic monitoring and by internal cardiotocography after rupture of the membranes. The heart rates of both twins were recorded concomitantly. After the delivery of the first twin and before rupture of the second amniotic sac the lie of the second fetus was assessed clinically and confirmed by real-time ultrasonography.

With the fetus in breech presentation an assisted breech delivery was performed. Signs of acute fetal distress at this stage of labor or inadequate progress despite controlled oxytocin drip (5 IU/500 ml of isotonic saline solution, maximal rate of 3 ml/min) prompted the performance of a total breech extraction. Artificial rupture of the second amniotic sac was undertaken as late as possible.

If the second twin was in an oblique or transverse lie after delivery of the first twin, internal version and total breech extraction were performed with the patient under general anesthesia or with epidural analgesia already established. In all cases of vaginal delivery a lateral episiotomy was performed. Vaginal delivery or cesarean section was performed by a resident physician with chief resident and/or senior obstetric staff supervision. Anesthesia and pediatric house staff provided support. Maternal outcome was judged by duration of hospitalization, febrile morbidity, need for blood transfusions, and operative morbidity.

Table I. Presentations of twins at admission

	Allocated route of delivery			
	Vaginal (n = 33)*		*Cesarean section (n = 27)*	
	n	%	*n*	%
Presentation				
Vertex-breech	21	60.6	18	66.7
Vertex-transverse	12	39.4	9	33.3
Indication for admission				
Spontaneous labor or ruptured membranes	29	87.9	22	81.5
Induced labor (postdates)	4	12.1	5	18.5

*Note that six pairs of twins delivered in a mode different from that determined by the study protocol are included in the vaginal delivery group.

The infants were examined immediately by a pediatrician not involved in the study who determined the Apgar scores and fetal weights. Further pediatric management was uninfluenced by the study and was undertaken according to neonatal performance. A cranial ultrasonic examination was performed routinely in infants with birth weights <1500 gm and whenever the test was clinically indicated. Parameters of neonatal outcome included Apgar scores, birth trauma, and neonatal morbidity and mortality.

Statistical analysis. Statistical evaluation of the data was accomplished by the paired and unpaired *t* test, the χ^2 test (with Yates' correction), and the Fisher exact test where applicable. Differences were considered to be statistically significant at $p < 0.05$.

Results

The study population comprised 60 consecutive pairs of twins in the vertex-breech or vertex-transverse presentation before delivery. Twenty-seven women were allocated to the cesarean section group and 33 to the vaginal delivery group. The presentations of the twins

Table II. Neonatal data of twins delivered according to primary allocation

	First twin		*Second twin*	
	Vaginal delivery (n = 27)	*Cesarean section (n = 27)*	*Vaginal delivery (n = 27)*	*Cesarean section (n = 27)*
Birth weight (gm)				
Mean ± SD	2477 ± 370	2533 ± 423	2459 ± 510	2484 ± 632
Range	1800-3065	1525-3360	1400-3330	1300-3550
Apgar score at 1 min		p < 0.00006*		
1-4	0	2	5	4
5-7	1	2	9	7
8-10	26	23	13	16
Apgar score at 5 min			p = 0.03*	
1-4	0	0	0	0
5-7	0	1	1	1
8-10	27	26	26	26
Hospitalization (days)			p < 0.0001†	
Mean ± SD	7.6 ± 8.6	10.1 ± 6.3	8.0 ± 8.1	13.1 ± 12.1
Range	3-35	5-36	3-39	5-60
Infants discharged on schedule‡ (No.)	16 (59.3%)	11 (40.7%)	13 (48.1%)	10 (37%)
Hypoglycemia	1	2	2	3
Transient tachypnea	1	2	0	2
Secondary apnea	0	0	2	0
Hyperbilirubinemia	3	4	5	5

*Fisher's exact test for >7 and ≤7.

†$t = 5.9$, degrees of freedom = 52.

‡Discharged at 3 or 4 days for vaginal delivery group and at 6 or 7 days for cesarean section group.

at admission and the indications for admission are shown in Table I. All inductions of labor were performed because of postdatism (after completed 41 weeks of gestation). Two women randomized for vaginal delivery subsequently underwent cesarean section (one because of inadequate progress of labor and another because of heart rate monitoring of the second twin suggesting fetal distress). In four cases in the vaginal delivery group, the second twin changed from transverse to vertex presentation spontaneously after delivery of the first twin (Fig. 1). Five second twins in breech presentation were delivered by total breech extraction because of inadequate descent (one), fetal distress suspected by fetal heart rate monitoring (two), or prolapse of the cord of the second twin after rupture of the membranes (two). In eight parturient women uterine and cervical examinations after internal version and total breech extraction did not reveal any uterine or cervical tears. Delivery of the second twin was performed with the patient under general anesthesia in five of these patients and under epidural analgesia in the remaining three. All women allocated to the abdominal delivery group underwent a transverse lower-segment cesarean section. General anesthesia was used in 12 parturient women and epidural analgesia in 15.

The Apgar scores of the firstborn and second-born twins as well as neonatal data of all deliveries performed according to the allocation protocol are shown in Table II. Birth weights did not differ significantly between the groups and between the firstborn and second-born twins. The 1-minute Apgar scores but not the 5-minute scores of the second-born twins in both groups were significantly lower when compared with those of the firstborn infants. No difference was found between the 1- and 5-minute Apgar scores of the second twins of both groups. The duration of hospitalization was longer in the cesarean section group, but these data were influenced by the prolonged hospitalization of the mothers after abdominal delivery. Mothers and infants are usually discharged 3 to 4 days after vaginal delivery and 6 to 7 days after cesarean section. Thus the incidence of late discharge did not differ significantly between the twins in the different study groups. The incidence of neonatal morbidity was similar in all groups. The data of the six pairs of infants delivered in a mode different from that determined by the study protocol are shown in Table III.

No case of birth trauma to any neonate was recorded. In three infants with birth weights <1500 gm a routine ultrasound examination of the skull did not reveal any abnormal findings. No case of intraventricular hemorrhage was diagnosed and all of the infants were well on discharge.

A significantly higher incidence of maternal febrile

Table III. Neonatal data of six pairs of twins delivered in a mode different from that determined by study protocol

	Vaginal delivery		*Cesarean section*	
	First twin (n = 4)	*Second twin (n = 4)*	*First twin (n = 2)*	*Second twin (n = 2)*
Weight (gm)				
Mean ± SD	2352 ± 188	2150 ± 609	3200 ± 141	2750 ± 353
Range	2100-2540	1400-2740	3300-3100	3000-2500
Apgar score at 1 min				
1-4	0	0	0	0
5-7	0	2	1	1
8-10	4	2	1	1
Apgar score at 5 min				
1-4	0	0	0	0
5-7	0	0	0	1
8-10	4	4	2	1
Hospitalization (days)				
Mean ± SD	3	9 ± 9.3	8 ± 1.4	8 ± 1.4
Range	3	3-15	7-9	7-9
Total neonatal morbidity	0	0	0	0

morbidity was observed in the cesarean section group (Table IV). Gestational age at delivery and nulliparity were not significantly different in either study group. Of the eight women who underwent internal version, total breech extraction, and subsequent uterine revision, two had postpartum fever.

Comment

For the last decade obstetricians have performed an increasing number of cesarean sections to deliver second twins in nonvertex presentations.[8, 11] The presented results suggest that in twins with vertex-breech or vertex-transverse presentations after the thirty-fifth week of gestational age the neonatal outcome of the second twin was not significantly influenced by the route of delivery. A low incidence of perinatal morbidity was noted in both groups. This favorable outcome may be related to the antenatal diagnosis of all twins, to the careful intrapartum monitoring of both fetuses, and to the fact that only otherwise uncomplicated pregnancies were included in the study. Antenatal diagnosis of a twin pregnancy is critical for adequate intrapartum cardiotocographic monitoring of both fetuses and the early detection of fetal distress. The poorer outcome for second twins in other studies may have been related to lack of early diagnosis and adequate intrapartum surveillance.[6, 7, 12] Comparable results have been reported in a retrospective study with obstetric management similar to the protocol presented.[13] It is noteworthy that despite the measures taken the 1-minute Apgar scores of the second twins were significantly lower than the scores of the first twin, irrespective of the mode of delivery. We attribute the lack of major birth trauma to the fact that all internal versions were performed with amniotic membranes unruptured and with anesthetic-analgesic medication. The low incidence of induced labor might explain the relatively low incidence of cesarean section for the parturient patients allocated to the vaginal delivery group (two of 33).

Table IV. Maternal data (delivery according to protocol)

	Vaginal delivery (n = 27)	*Cesarean section (n = 27)*
Weeks' gestation at delivery		
Mean ± SD	37.7 ± 1.6	37.5 ± 1.5
Range	35-41	35-41
Age (yr)		
Mean ± SD	30.3 ± 4.3	29.8 ± 5.2
Range	21-40	21-37
Nulliparity (No.)	6 (22.2%)	7 (26.9%)
Postpartum hospitalization (days)		
Mean ± SD*	4.9 ± 2.9	8 ± 2
Range	3-13	6-14
Patients discharged on schedule†	18 (66.7%)	13 (48.1%)
Maternal febrile morbidity‡	3 (11.1%)	11 (40.7%)
Maternal anemia (need for blood transfusion)	2	3

*$p < 0.0001$ ($t = 4.49$, degrees of freedom = 52).

†Discharged at 3 or 4 days for vaginal delivery group and at 6 or 7 days for cesarean section group.

‡$p = 0.01$ (Fisher's exact test).

Only parturient women beyond 35 weeks' gestation were considered for the study. The rationale for this criterion was based on the experience of others who failed to demonstrate an increased risk for the second nonvertex twin delivered vaginally with a birth weight of >1500 gm.[9, 14] All of the neonatal deaths and most of the intraventricular hemorrhages reported by others

occurred in infants of lower birth weights.[8, 9] The limit of 35 gestational weeks seems quite reliable as only three infants had a birth weight of <1500 gm. Reliance on prenatal ultrasonic estimation of fetal growth should assist the obstetrician to exclude very low birth weight infants from the potential risks of vaginal delivery.

In this study the incidence of maternal morbidity after cesarean section compared with that after vaginal delivery was high and compares to the incidence in similarly conducted studies.[15] This point is even further stressed if one considers the relatively low incidence of maternal morbidity in women undergoing internal version and uterine revision. Women with abdominal delivery were discharged from the hospital later than patients with vaginal delivery (6 to 7 days as compared to 3 to 4 days), and 48.1% of the parturient women in the cesarean section group compared with 66.7% of the vaginal delivery group, left the hospital at the appropriate time (nonsignificant difference) (Table IV).

In four of 12 pairs of twins with vertex-transverse presentations, the second fetus spontaneously changed to vertex presentation during the course of vaginal delivery. These findings and the good results reported by others undertaking external cephalic version after the birth of the first twin[16] suggest that during vaginal delivery of vertex-nonvertex twins some second-born fetuses may present in vertex position at the time of expulsion. There is general agreement that vaginal delivery is appropriate for vertex-vertex–presenting twins.[3, 13]

The favorable outcomes of the second-born twins delivered vaginally as well as the established maternal risks associated with cesarean section support the contention that in selected twin pregnancies with vertex-breech or vertex-transverse presentations at a gestational age of 35 weeks or more, a vaginal delivery can be performed without increased risk to either mother or infant. We appreciate that because of the rarity of adverse outcome in this study the possibility of a type 2 statistical error that can be attributed to the sizes of the subpopulation should be taken into account. We shall continue this study to expand the groups and to strengthen the data. In recent articles the authors[8, 9, 11, 13] voiced their hope of settling the controversy on the appropriate management of the second nonvertex twin by randomized prospective studies. Our results suggest similar neonatal outcome irrespective of the route of delivery of these infants. It is hoped that the presented data will encourage others to conduct similar prospective studies or multicenter studies in order to substantiate our findings in larger study groups.

REFERENCES

1. Taylor ES. Editorial. Obstet Gynecol Surv 1983;38:272.
2. Kelsick F, Minkoff H. Management of the breech second twin. Am J Obstet Gynecol 1982;144:873.
3. Cetrulo CL, Ingardia CJ, Sbarra AJ. Management of multiple gestation. Clin Obstet Gynecol 1980;23:533.
4. Taylor ES. Editorial. Obstet Gynecol Surv 1976;31:535.
5. Kauppila A, Jouppila P, Koivisto M, Moilanen M, Ylirkola O. Twin pregnancy: a clinical study of 355 cases. Acta Obstet Gynecol Scand 1975;54(suppl):5.
6. Ho SK, Wu PYK. Perinatal factors and neonatal morbidity in twin pregnancy. Am J Obstet Gynecol 1975;122:979.
7. Ware HH. The second twin. Am J Obstet Gynecol 1971; 100:865.
8. Acker D, Lieberman M, Holbrook H, et al. Delivery of the second twin. Obstet Gynecol 1982;59:710.
9. Chervenak FA, Johnson, RE, Berkowitz RL, Grannum P, Hobbins JC. Is routine cesarean section necessary for vertex-breech and vertex-transverse twin gestations? Am J Obstet Gynecol 1984;148:1.
10. Rayburn WF, Lavin JP, Miodovnik M, Varner MW. Multiple gestation: time interval between delivery of the first and second twins. Obstet Gynecol 1984;63:502.
11. Taylor ES. Editorial. Obstet Gynecol Surv 1984;39:498.
12. Farouqui MO, Grossman JH, Shannon RA. A review of twin pregnancy and perinatal mortality. Obstet Gynecol Surv 1973;28:144.
13. Chervenak FA, Johnson RE, Youcha S, Hobbins JC, Berkowitz RL. Intrapartum management of twin gestation. Obstet Gynecol 1985;65:119.
14. Barrett JM, Staggs SM, van Hooydonk JE, Growdon JH, Killam AP, Boehm FH. The effect of type of delivery upon neonatal outcome in premature twins. Am J Obstet Gynecol 1982;143:360.
15. Collea JV, Chein C, Quilligan WJ. The randomized management of term frank breech presentation: a study of 208 cases. Am J Obstet Gynecol 1980;137:235.
16. Chervenak FA, Johnson RE, Berkowtiz RL, Hobbins JC. Intrapartum external version of the second twin. Obstet Gynecol 1983;62:160.

Natalins Rx

Multivitamin and Multimineral Supplement with 1 mg Folic Acid

The Only Prenatal Vitamin with No Artificial Color or Flavor from Synthetic Sources

The Recipe for Prenatal Good Health

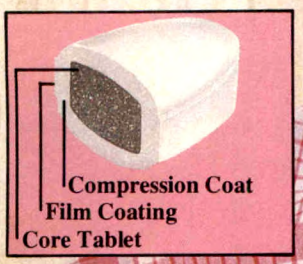
Good things are made from the finest ingredients. And the ingredients for maintaining good prenatal health make NATALINS Rx a standout . . . inside and out.

NATALINS Rx is the first and only prescription vitamin and mineral supplement with "natural" color and flavor. It contains all the mandatory and optional vitamins and minerals, plus copper and zinc, both important in the metabolic process.

Unique NATALINS Rx Construction Aids Compliance and Maintenance of Good Health

An elliptical tablet shape and a cellulosic film coating that becomes slippery when wet allows for easier, more comfortable swallowing—aiding patient compliance. Beneath the special coating, a central "core tablet" stores iron for delayed release, reducing the risk of stomach upset.

NATALINS Rx . . . the "Natural" Choice for Prenatal Good Health.



NATALINS® Rx

Ingredients	Natalins Rx	
VITAMINS	**Units**	**% RDA**
Vitamin A, IU	8000	100
Vitamin D, IU	400	100
Vitamin E, IU	30	100
Vitamin C, mg	90	150
Folic Acid, mg	1	125
Thiamine (B_1), mg	2.55	150
Riboflavin (B_2), mg	3	150
Niacin, mg	20	100
Vitamin B_6, mg	10	400
Vitamin B_{12}, mcg	8	100
Biotin, mg	0.05	16
Pantothenic Acid, mg	15	150
MINERALS		
Calcium, mg	200	15
Iodine, mcg	150	100
Iron, mg	60	333
Magnesium, mg	100	22
Copper, mg	2	100
Zinc, mg	15	100
Artificial color	none	
Artificial flavor	none	

Natalins Rx is a multivitamin, multimineral supplement for pregnant or lactating women. Daily dose: 1 tablet or as prescribed.
Contraindications: Natalins Rx should not be prescribed in patients with hemochromatosis or patients with Wilson's disease.
Caution: Pernicious anemia should be excluded before using this product. In patients with kidney stones, the calcium content of Natalins Rx tablets should be considered before prescribing.
Supplied: Bottles of 100 and 1,000.

Mead Johnson LABORATORIES
Mead Johnson & Company
Evansville, Indiana 47721 U.S.A.

©1985 Mead Johnson & Company • Evansville, Indiana 47721 U.S.A.
N-1-85

In intra-abdominal infection...

Consider

Fact No. 1

Bacteroides fragilis is the major anaerobic pathogen[1]

"The most common anaerobic organism is B fragilis, isolated in 80 to 95 percent of patients with colorectal perforation."[2]

Fact No. 2

Against *B fragilis*, CEFOTAN has equal in vitro activity to cefoxitin*[3-10]

Gorbach, Quintiliani, Neu, and others have studied a total of more than 900 isolates of *B fragilis* and determined that CEFOTAN and cefoxitin have equal in vitro activity.*[3-10]

Fact No. 3

Intra-abdominal infection is polymicrobial

Tally and Gorbach report that *B fragilis* is the major anaerobic pathogen, while *Escherichia coli* is the primary aerobic isolate.[11]

Fact No. 4

CEFOTAN has superior gram-negative activity compared with cefoxitin[12]

"...spectrum of activity [of CEFOTAN] is distinguished by having the same anti-anaerobe[†] effect as cefoxitin coupled with enhanced activity against Esch. coli and other Enterobacteriaceae."[13]

—C.J.L. Strachan—

*In vitro activity does not necessarily correlate with in vivo effectiveness.

†Most strains of *B distasonis*, *B ovatus*, and *B thetaiotaomicron* are resistant to CEFOTAN.

© 1986 ICI Americas Inc

The Facts

Fact No. 5

CEFOTAN is cost-effective

Twice-daily CEFOTAN can save 45% in treatment costs and single-dose prophylaxis with CEFOTAN saves 63% when compared with a three-dose regimen of cefoxitin.‡

Conclusion

The facts now favor CEFOTAN

CEFOTAN is an ideal agent in the treatment of intra-abdominal infections due to susceptible pathogens including *Bacteroides* species such as *B fragilis*, but not *B distasonis*, *B ovatus*, or *B thetaiotaomicron*.

As active as cefoxitin against *Bacteroides fragilis*—the most common anaerobe in intra-abdominal sepsis—CEFOTAN provides superior gram-negative coverage and is particularly active against *E coli* and other common gram-negative pathogens.

In intra-abdominal infection due to indicated organisms Switch to

CEFOTAN® IM/IV

(cefotetan disodium)

The cost-effective replacement for cefoxitin

Please see the following page for brief summary of prescribing information.

‡Based on overall costs of antibiotic preparation and delivery[14] for comparable regimens: Treatment—CEFOTAN, 2 g × 2; cefoxitin,[15] 2 g × 4. Prophylaxis—CEFOTAN 2 g × 1; cefoxitin,[15] 2 g × 3.

CEFOTAN IM/IV
(cefotetan disodium)

For Intravenous or Intramuscular Use

BRIEF SUMMARY

INDICATIONS AND USAGE
TREATMENT: CEFOTAN® (cefotetan disodium) is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:

Urinary tract infections caused by *E coli*, *Klebsiella* species (including *K pneumoniae*), *Proteus mirabilis* and *Proteus* sp (which may include the organisms now called *Proteus vulgaris*, *Providencia rettgeri*, and *Morganella morganii*).

Lower respiratory tract infections caused by *Streptococcus pneumoniae* (formerly *D pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K pneumoniae*), and *E coli*.

Skin and skin structure infections caused by *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis*,* *Streptococcus pyogenes* and *Streptococcus* species (excluding enterococci), and *E coli*.

Gynecologic infections caused by *Staphylococcus aureus*† (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis*,* *Streptococcus* species (excluding enterococci), *E coli*, *Proteus mirabilis*, *Neisseria gonorrhoeae*, *Bacteroides* species (excluding *B distasonis*, *B ovatus*, *B thetaiotaomicron*), *Fusobacterium* species,† and gram-positive anaerobic cocci (including *Peptococcus* and *Peptostreptococcus* species†).

Intra-abdominal infections caused by *E coli*, *Klebsiella* species (including *K pneumoniae*†), *Streptococcus* species (excluding enterococci), and *Bacteroides* species (excluding *B distasonis*, *B ovatus*, *B thetaiotaomicron*).

Bone and joint infections caused by *Staphylococcus aureus*.†

***NOTE:** Some strains of *Staphylococcus epidermidis* are resistant to cefotetan.

†Efficacy for this organism in this organ system was studied in fewer than ten infections.

PROPHYLAXIS: The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (eg, cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS
CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS
Before therapy with CEFOTAN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefotetan disodium, cephalosporins, penicillins, or other drugs. This product should be given cautiously to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to CEFOTAN occurs, discontinue the drug. Serious acute hypersensitivity reactions may require epinephrine and other emergency measures.

Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with antibiotic use.

Treatment with broad-spectrum antibiotics may alter normal flora of the colon and may permit overgrowth of clostridia. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin in vitro.

Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte, and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance, or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C difficile*. Other causes should also be considered.

PRECAUTIONS
GENERAL: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity. Those at risk include patients with renal or hepatic impairment, or poor nutritional state. Prothrombin times should be monitored in patients at risk and exogenous Vitamin K administered as indicated.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

INFORMATION FOR PATIENTS: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.

DRUG INTERACTIONS: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycoside antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

DRUG/LABORATORY TEST INTERACTIONS: A false positive reaction for glucose in urine may occur with Benedict's or Fehling's solution.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels of Jaffe reaction and produce false increases in the levels of creatinine reported.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approx 8-16 times the usual adult human dose) on days 6-35 of life (thought to be developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approx 2-4 times the usual human dose), only 1 of 10 treated animals was affected, and the degree of degeneration was mild. Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.

USAGE IN PREGNANCY: Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

USAGE IN NURSING MOTHERS: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.

PEDIATRIC USE: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS
In clinical studies the following adverse effects were considered related to CEFOTAN therapy.

Gastrointestinal symptoms occurred in 1.5 percent of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700).

Hematologic laboratory abnormalities occurred in 1.4 percent of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300).

Hepatic enzyme elevations occurred in 1.2 percent of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).

Hypersensitivity reactions were reported in 1.2 percent of patients and included rash (1 in 150) and itching (1 in 700).

Local effects were reported in less than one percent of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500).

DOSAGE AND ADMINISTRATION
TREATMENT: The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6* grams	3 g every 12 hours IV

*Maximum daily dosage should not exceed 6 grams.

PROPHYLAXIS: To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.

IMPAIRED RENAL FUNCTION: When renal function is impaired, a reduced dosage schedule must be employed. The following dosage guidelines may be used.

DOSAGE GUIDELINES FOR PATIENTS WITH IMPAIRED RENAL FUNCTION

Creatinine Clearance mL/min	Dose	Frequency
>30	Usual Recommended Dose*	Every 12 hours
10-30	Usual Recommended Dose*	Every 24 hours
<10	Usual Recommended Dose*	Every 48 hours

*Dose determined by the type and severity of infection, and susceptibility of the causative organism.

Cefotetan is dialyzable and it is recommended that for patients undergoing intermittent hemodialysis, one-quarter of the usual recommended dose be given every 24 hours on days between dialysis and one-half the usual recommended dose on the day of dialysis.

HOW SUPPLIED
CEFOTAN is a dry, white to pale yellow powder supplied in vials containing cefotetan disodium equivalent to 1 g and 2 g cefotetan activity for intravenous and intramuscular administration. The 1 g dose is available in 10 mL and 100 mL vials, and the 2 g dose is available in 20 mL and 100 mL vials. The vials should not be stored at temperatures above 22°C and should be protected from light.
1 g in 10 mL vial (NDC 0038-0376-10)
2 g in 20 mL vial (NDC 0038-0377-20)
1 g in 100 mL vial (NDC 0038-0376-11)
2 g in 100 mL vial (NDC 0038-0377-21)

Manufactured for



STUART PHARMACEUTICALS
Division of ICI Americas Inc
Wilmington, Delaware 19897

Rev E 3/86

63967-02

References: 1. Gorbach SL: Bacteroides species, in Mandel GL, Douglas RG Jr, Bennett JE (eds): *Principles and Practice of Infectious Diseases*, ed 2. New York, John Wiley & Sons, 1985, p 1370. 2. Simmons RL, Howard RJ, Henriksen AI (eds): *Surgical Infectious Diseases*. New York, Appleton-Century-Crofts, 1982, p 922. 3. Souza Dias MB, Jacobus NV, Tally FP, Gorbach SL: Activity of cefotetan against anaerobic bacteria. *Diagn Microbiol Infect Dis* 1986;4:359-363. 4. Data on file, Stuart Pharmaceuticals, Wilmington, Delaware. 5. Neu HC, Chin NX: The activity and beta-lactamase stability of cefotetan compared to other beta-lactam antibiotics. *Chemioterapia* 1985;4:271-277. 6. Hennessey TD: Cefotetan: A compilation of susceptibility data of clinical isolates, in Lode H, Periti P, Strachan CJL (eds): *Cefotetan: A Long-Acting Antibiotic for Polymicrobial Infections*. Proceedings of a Symposium Held in Association With the 4th Mediterranean Congress of Chemotherapy, Rhodes, Greece, 1984. New York, Churchill Livingstone, 1985, pp 14-22. 7. Aldridge KE, Sanders CV, Janney A, et al: Comparison of the activities of penicillin G and new β-lactam antibiotics against clinical isolates of *Bacteroides* species. *Antimicrob Agents Chemother* 1984;26:410-413. 8. Watt B, Brown FV: The comparative *in-vitro* activity of cefotetan against anaerobic bacteria. *J Antimicrob Chemother* 1985;15:671-677. 9. Ruckdeschel G: Activity *in vitro* of cefotetan against non-sporing anaerobes: A comparative study. *J Antimicrob Chemother* 1983;11(suppl A):117-124. 10. Werner H: Inhibitory activity of cefotetan and other β-lactams against anaerobes. *J Antimicrob Chemother* 1983;11(suppl A):107-115. 11. Tally FP, Gorbach SL: Therapy of mixed anaerobic-aerobic infections: Lessons from studies of intra-abdominal sepsis. *Am J Med* 1985;78(suppl 6A):145-153. 12. Ayers LW, Jones RN, Barry AL, et al: Cefotetan, a new cephamycin: Comparison of in vitro antimicrobial activity with other cephems, β-lactamase stability, and preliminary recommendations for disk diffusion testing. *Antimicrob Agents Chemother* 1982;22:859-877. 13. Strachan CJL: Postscript, in Lode H, Periti P, Strachan CJL (eds): *Cefotetan: A Long-Acting Antibiotic for Polymicrobial Infections*. Proceedings of a Symposium Held in Association With the 4th Mediterranean Congress of Chemotherapy, Rhodes, Greece, 1984. New York, Churchill Livingstone, 1985, p 289. 14. McCue JD: *Cost-Conscious Use of the Cephalosporins*. New York, Raven Health Care Communications, 1985, p 37. 15. Direct price from *Drug Topics Red Book*, ed 90. Oradell, NJ, Medical Economics Co, 1986, p 378.

FOR VULVOVAGINAL CANDIDIASIS

Think Cream



GOOD NEWS ABOUT MALPRACTICE!

Informed Consent™ Systems was developed by doctors and lawyers to help eliminate doctor/patient litigation.

The **Informed Consent™ System** is:

- A *Videotape* designed to inform a patient about surgical procedures in an easy-to-understand format.
- A *Legal Support Service* designed to supply materials which show that the duty to obtain [the patient's] informed consent was discharged.

—SPANISH FORMAT AVAILABLE—

Features:

- Videotapes explain surgery in a clear and uncomplicated manner.
- Videotapes explain risks and complications but are not designed to scare patients.
- Videotapes use diagrams and illustrations instead of actual photographs.
- Legal Support Service stores evidential materials and provides access for later courtroom use.

Informed Consent™ Systems specialty areas:

◆ obstetrics/gynecology ◆ orthopedic ◆
◆ otolaryngology ◆ urology ◆
◆ endarcardiology/vasiculary ◆
◆ general surgery ◆

For more information, or to order, call:
1-800-FILMTEC
VA residents call: **703-471-6891**
distributed by Filmtec, Inc. reston, va. 22090
©1986. Filmtec, Inc.

OBSTETRICIAN-GYNECOLOGIST

24 member multispecialty clinic seeking third board certified or eligible OB/GYN physician in Bemidji, Minnesota. Excellent opportunity to join a high quality medical community in beautiful north woods and lake country of Northern Minnesota. Office ultrasound and colposcopy available. Immediate full clinical practice of interesting obstetrics and gynecological patients. Contact:

C.C. Lowery, Administrator
Bemidji Clinic, Ltd.
1233 34th Street NW
Bemidji, Minnesota 56601
218-751-1280

MATERNAL FETAL MEDICINE

Board eligible/certified Perinatologist as Associate Director for Perinatal Center in 423 bed teaching hospital serving high-risk population. Opportunity for clinical practice, clinical research, medical student and residency teaching, maternal transport care, private practice, ultrasonography and outreach education. Position available July, 1987. Salary and academic rank commensurate with experience. Opportunity for young qualified individual to further develop Perinatal Center and Division of Maternal-Fetal Medicine.

Send inquiry and CV to:

Washington C. Hill, M.D., FACOG
Creighton University Medical Center
The Perinatal Center
601 North 30th Street, Suite 4810
Omaha, Nebraska 68131-2197
or call (402) 280-4431
1-800-228-7212, Ext. 4431

FOR VULVOVAGINAL CANDIDIASIS

Think 3 Days*



Boston University School of Medicine
Dept. of Obstetrics & Gynecology

presents

A REVIEW COURSE AND UPDATE IN OBSTETRICS AND GYNECOLOGY—1987

Course Director
Leonard J. Cibley, M.D.

A distinguished faculty will emphasize controversial topics and management of patients in broad areas of obstetrical and gynecological practice. This program should prove of interest to the practicing obstetrician-gynecologist, pediatrician, resident-in-training, as well as to the nurse-midwife and nurse practitioner.

March 30 — April 1, 1987

Royal Sonesta Hotel
Cambridge, Massachusetts

For further information, contact

Dept. of Continuing Medical Education
Boston University School of Medicine
80 E. Concord St.
Boston, MA 02118
(617) 638-4605.

OB/GYN
Central New Jersey

BC/BE physician who has recently completed or is completing a university affiliated residency wanted to join a solo practitioner in central New Jersey.

Association leading to partnership, malpractice insurance paid. Competitive salary.

Send C.V. and letter to:

Charles M. Fleisch, D.O., FACOG
415 Avenel Street
Avenel, NJ 07001

or call:
201-636-2122

Join the Group in Minneapolis.

Outstanding career opportunity combining superior care with professional growth. Join progressive department of 20 physicians, 12 nurse-midwives and 6 ob/gyn nurse practitioners delivering 300 babies each month and providing family planning, infertility, high-risk obstetrics, laser surgery, colposcopy, ultrasonography, laparoscopy and maternal/fetal medicine in one of America's leading metropolitan areas.

Comprehensive benefits including professional liability, four weeks vacation, two weeks conference leave, sick leave, medical coverage, dental coverage, life insurance, disability and retirement plans. Opportunities for teaching and university affiliation. Please send c.v. to: **M. Aksoy, M.D., Chairman, Department of Obstetrics and Gynecology; GROUP HEALTH, INC.; 2829 University Ave. S.E., Suite 220; Minneapolis, MN 55414.**

Maternal-Fetal Medicine

Board certified/eligible PERINATOLOGISTS required for large volume obstetric services (3,000-6,000 deliveries per year).

Opportunities exist both to join and create high risk obstetric services at various Kaiser Permanente Medical Care Centers located in Southern California. University affiliations and clinical research is encouraged.

Excellent benefits are offered including liability coverage, medical and dental coverage, vacation and sick leave, continuing education and retirement program. Partnership consideration available after two years full time employment.

Please write, including your curriculum vitae and three professional references to:

KAISER PERMANENTE
SOUTHERN CALIFORNIA
PERMANENTE MEDICAL GROUP

William G. McCormick
Physician Recruitment
Dept. 41B
Walnut Center
Pasadena, CA 91188
(714)829-5365
Equal Opportunity Employer M/F/H

FOR VULVOVAGINAL CANDIDIASIS

Think

vaginal cream 2.0%

Femstat®

(butoconazole nitrate)





FAST, SOOTHING RELIEF
Complete coverage–
and minimal vaginal leakage![1]

3-DAY THERAPY
As effective as miconazole cream–
in only 3 days' time!

3 DISPOSABLE APPLICATORS
Patient convenience and satisfaction–
for enhanced compliance!

*In non-pregnant patients, 3-day treatment is recommended. Treatment may be extended an additional 3 days, if necessary.

1. Droegemueller W, Adamson DG, Brown D et al: Three-day treatment with butoconazole nitrate for vulvovaginal candidiasis. *Obstet Gynecol* 64:530-534, 1984.

Please see brief summary of prescribing information on next page.

The Only 3-Day Cream*

Think

vaginal cream 2.0%

Femstat®

(butoconazole nitrate)

The Only 3-Day Cream

Brief Summary

INDICATIONS:
FEMSTAT® (butoconazole nitrate) vaginal cream 2.0% is indicated for local treatment of vulvovaginal mycotic infections caused by Candida species. Confirm the diagnosis by KOH smears and/or cultures. FEMSTAT can be used with oral contraceptives and antibiotics. It is effective in non-pregnant women and during the second and third trimesters of pregnancy.

CONTRAINDICATIONS:
FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

PRECAUTIONS:
General:
If clinical symptoms persist, repeat microbiological tests to rule out other pathogens and confirm the diagnosis. Discontinue drug if sensitization or irritation occurs.

Information for the Patient:
Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis:
Animal studies have not been done.

Mutagenesis:
Mutagenicity studies were negative.

Impairment of Fertility:
Animal studies showed no impairment of fertility.

Pregnancy Category C:
Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers:
Use with caution.

Pediatric Use:
Safety and efficacy have not been established.

ADVERSE REACTIONS:
Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

DOSAGE AND ADMINISTRATION:
Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

CAUTION:
Federal law prohibits dispensing without prescription.

December 1985

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

© 1986 Syntex Laboratories, Inc.

OB/GYN PRACTICE OPPORTUNITY

In La Junta, Colorado, community of 10,000 with a HSA of 35,000. 61 - bed modern, full - service, JCAH - accredited hospital with 27 - member active multispecialty medical staff. Income guarantee. Excellent fishing, hunting, skiing.

CONTACT:

W. Holm, M.D.
1100 Carson
La Junta, CO 81050
303 384-7734

REPRODUCTIVE ENDOCRINOLOGIST

University of Wisconsin, Milwaukee Clinical Campus, Department of Obstetrics and Gynecology, is seeking a full-time board eligible or board certified reproductive endocrinologist for July, 1987. Position involves patient care and teaching with opportunities for research. Administrative role includes direction of **in vitro** fertilization program. Send curriculum vitae to:

Phillip R. Hamilton, M.D.
Chairman, Department of
Obstetrics and Gynecology
Mount Sinai Medical Center
950 North 12th Street
Milwaukee, Wisconsin, 53233

An equal opportunity employer

Predictors of vaginal delivery in patients with a previous cesarean section, who require oxytocin

Richard K. Silver, M.D., and Ronald S. Gibbs, M.D.
San Antonio, Texas

Prospective analysis of 98 consecutive patients at term pregnancy with one previous cesarean section, who received oxytocin during a trial of labor (34 inductions, 64 augmentations), was undertaken to identify specific factors associated with successful vaginal delivery. The overall vaginal delivery rate was 59.2%. Comparing route of delivery in the induction and augmentation groups separately revealed no significant differences in maternal height, weight, or parity, duration of membrane rupture, length of oxytocin treatment or maximum dose, cervical examination on admission or before oxytocin treatment, or use of conduction anesthesia. A previous vaginal delivery favored repeat vaginal delivery in patients with augmentation while a nonrecurrent indication was significantly associated with vaginal delivery in all patients. After the beginning of oxytocin augmentation, the cervical dilatation rate was 1.82 cm/hr in patients delivered vaginally, compared with 0.18 cm/hr in those requiring cesarean section ($p < 0.001$). Any cervical dilatation during the first 2 hours of augmentation was associated with more frequent vaginal delivery: 24 of 40 vaginal deliveries (60%) versus six of 24 cesarean sections (25%, $p < 0.01$). Discriminant analysis correctly identified route of delivery in 85.3% of those with induction and 87.5% of patients with augmentation. During a trial of labor, oxytocin induction or augmentation is effective in a majority of patients. Furthermore, an early response during augmentation is of predictive value when such patients are being managed. (AM J OBSTET GYNECOL 1987;156:57-60.)

Key words: Oxytocin, trial of labor, cervical dilatation rate

Although the revised recommendations of the American College of Obstetricians and Gynecologists[1] support oxytocin use during a trial of labor, wide application of this practice has not been observed. Many reviews of vaginal delivery after cesarean section include an analysis of oxytocin use.[2-8] In general, patients receiving oxytocin in these series were delivered vaginally as often as women laboring spontaneously. However, less than half of the women in each of these retrospective studies actually received oxytocin (range 2% to 41%). Also the indications for oxytocin and/or the methods of administration were infrequently defined. Finally all of these reports introduce the potential bias of patient self-selection (for trial of labor), which may impact on route of delivery and overall success rate. The present prospective study was undertaken to determine whether oxytocin is effective for induction or augmentation in patients undergoing a trial of labor and to identify those factors predictive of successful vaginal delivery.

From the Division of Obstetrics, Department of Obstetrics and Gynecology, University of Texas Health Science Center.
Presented at the Sixth Annual Meeting of The Society of Perinatal Obstetricians, San Antonio, Texas, January 30–February 1, 1986.
Received for publication May 12, 1986; revised July 14, 1986; accepted July 17, 1986.
Reprint requests: Richard K. Silver, M.D., 333 E. Superior St., #438. Chicago, IL 60611.

Material and methods

One hundred consecutive patients with singleton pregnancies at term and a history of one low-transverse cesarean section, receiving intrapartum oxytocin, were included in this prospective study from March 1983 through February 1985. Thirty-four patients underwent induction of labor and 64 augmentations were performed. Indications for induction included spontaneous premature rupture of the membranes (n = 17), impending postdates (n = 6), pregnancy-induced hypertension (n = 5), diabetes mellitus (n = 5), and intra-amniotic infection (n = 1). All patients were delivered at Medical Center Hospital in San Antonio, Texas. Two patients received oxytocin during the second stage only and were excluded from further analysis. No other patient with a previous cesarean section, who required oxytocin, was excluded during this study interval.

Criteria for induction of labor included an obstetric indication for delivery, the absence of regular contractions, and pretreatment dilatation <3 cm, whereas labor augmentation required dilation ≥4 cm, regular uterine activity, and no cervical change during the preceding 1 hour. For induction, oxytocin was administered by doubling the initial dose of 1 mU/min every 30 minutes to a maximum dose of 64 mU/min; for augmentation, the same initial dose was increased by 1 mU/min every 30 minutes. All patients had intra-

Table I. Obstetric variables associated with successful trial of labor after induction

Obstetric variable	*n*	*Successful trial of labor*		
		No.	*%*	
No previous vaginal delivery	31	16	51.6	
≥1 Vaginal delivery	3	2	66.7	
Recurrent indication	24	9	37.5	*
Nonrecurrent indication	10	9	90.0	*
Present birth weight > past birth weight	23	11	47.8	
Past birth weight > present birth weight	11	7	63.6	

*p < 0.01.

Table II. Obstetric variables associated with successful trial of labor after augmentation

Obstetric variable	*n*	*Successful trial of labor*		
		No.	*%*	
No previous vaginal delivery	50	28	56.0	*
≥1 Vaginal delivery	14	12	85.7	*
Recurrent indication	38	19	50.0	*
Nonrecurrent indication	26	21	80.8	*
Present birth weight > past birth weight	39	19	48.7	†
Past birth weight > present birth weight	25	21	84.0	†

*p < 0.05.
†p < 0.01.

uterine pressure catheters placed before oxytocin administration, and Montevideo Units were calculated to assess response to oxytocin. After vaginal delivery, the integrity of the lower uterine segment was verified by examination in all cases.

Prenatal, intrapartum, and delivery variables were recorded for each patient. Analysis of data was accomplished with the Statistical Package for Social Sciences (X),[9] including χ^2 analysis for discrete variables and Student's *t* test (two-tailed) for continuous data and stepwise discriminant analysis.

Results

The overall vaginal delivery rate was 59.2% (inductions, 52.9%; augmentations, 62.5%). Selected obstetric variables associated with a successful trial of labor are summarized in Tables I and II. There were no significant differences in maternal height (expressed as either mean height or number of women <60 inches tall in each group), weight, or parity, when route of delivery was compared among all patients. In this same comparison, duration of membrane rupture, length of oxytocin treatment or maximum dose, cervical examination on admission or before oxytocin treatment, and use of conduction anesthesia were similar among both the induced and augmented groups. A positive association with previous vaginal delivery was limited to the augmented group (85.7% vaginal delivery rate with this history), while a nonrecurrent indication favored vaginal delivery in all patients. Nonrecurrent indications included malpresentation (n = 15), fetal distress (n = 11), pregnancy-induced hypertension (n = 6), multiple gestation (n = 3), and placenta previa (n = 1). Analysis of women with a lower birth weight at cesarean section than in the present pregnancy demonstrated that a larger infant during the current pregnancy did not preclude vaginal delivery. Overall, of the 62 women with larger infants, 30 were delivered vaginally. However, among patients with augmentation, a lighter infant during the current pregnancy favored a successful trial of labor (84.0% versus 48.7%, $p < 0.01$), and infants born by cesarean section were heavier than the neonates delivered vaginally (3548 versus 3280 gm, $p < 0.05$).

Table III. Cervical dilatation rates among patients with augmentation compared by route of delivery

Labor interval	*Dilatation rate (cm/hr, mean ± SD)*		*p*
	Cesarean (n = 24)	*Vaginal (n = 40)*	
Onset of active labor to oxytocin treatment	0.29 ± 0.24	0.39 ± 0.21	=0.08
Oxytocin treatment to complete dilatation or labor arrest	0.18 ± 0.21	1.82 ± 2.01	<0.001
First 2 hr of oxytocin treatment	0.18 ± 0.28	1.19 ± 1.23	<0.001

Although oxytocin was stopped or reduced more frequently among patients with augmentation requiring cesarean section, all patients demonstrated at least 150 Montevideo Units for two consecutive hours, without cervical change, before abdominal delivery. Reasons for reducing or stopping oxytocin included fetal heart rate abnormalities (n = 13), hypercontractility (n = 6), or both (n = 6).

Significant associations between progressive cervical effacement or descent of the presenting part and route of delivery were not identified. However, rates of cervical dilatation, before and after oxytocin treatment, were significantly different when compared by route of delivery. Three of these interval cervical dilatation rates are shown for the patients with augmentation in Table III. Before oxytocin treatment, the vaginal delivery group had a slightly increased rate of dilatation

($p = 0.08$). After augmentation, this same group had dilatation at 1.82 cm/hr for the remainder of active-phase labor, compared with only 0.18 cm/hr in those patients delivered by cesarean section ($p < 0.001$). Even during the first 2 hours of augmentation (when a maximum oxytocin dose of only 4 mU/min could be administered), patients destined to be delivered vaginally demonstrated significant cervical change. In contrast, dilatation rates during the first 2 hours of induction ($n = 34$) did not discriminate between route of delivery in that group of patients (vaginal, 0.30 cm/hr; cesarean, 0.22 cm/hr).

Table IV details the vaginal delivery rates as a function of cervical change during the first 2 hours of augmentation. Any change in dilatation during this period was associated with vaginal delivery in 75% of cases, while no change was associated with cesarean section in the same percentage of patients. Successively higher rates of dilatation enhanced the predictive value for vaginal delivery. Overall, in 24 of the 40 vaginal deliveries (60%), there was some degree of cervical change during this interval; this is compared with only six of the 24 cesarean sections (25%, $p < 0.01$).

Discriminant analysis was used to identify variables that predicted route of delivery among patients with both induction and augmentation. Initially both prospective (that is, known before oxytocin treatment) and retrospective (that is, known after delivery) variables were allowed to enter the model. Variables selected included cervical dilatation rates before oxytocin and in the first 2 hours after oxytocin, cervical examination (dilation) before oxytocin, active-phase dilatation rate, and maximum oxytocin dose. In this analysis, route of delivery was correctly identified in 29 of the 34 patients with induction (85.3%) and in 57 of the 64 patients with augmentation (89.1%). Next, only dilatation rates before oxytocin and in the first 2 hours after oxytocin were allowed to enter the model for the augmented group. These two prospective variables correctly identified 80% of the cesarean deliveries and 66.7% of those women with successful trials of labor.

Measures of morbidity included the incidence of antepartum and puerperal infection, intraoperative complications including uterine dehiscence, the need for blood transfusion, and any neonatal 5-minute Apgar score <7. Most complications were incurred by the patients with failed trials of labor. The diagnosis of intraamniotic infection was made in five women, three of whom were delivered by cesarean section. Nine of the 11 cases of endometritis were also encountered among cesarean section patients. All infections responded to appropriate antibiotic therapy. Two patients were noted to have uterine "windows" that had been asymptomatic during labor. These were repaired without complication at the time of cesarean section. Two other patients received a blood transfusion in the postoperative period. All neonatal 5-minute Apgar scores were ≥7.

Table IV. Cervical dilatation rate during the first 2 hours of augmentation and associated rates of vaginal delivery

Cervical dilatation rate (cm/hr)	*No. of patients with this rate (n = 64)*	*Patients delivered vaginally (%)*
≥0*	64	62.5
≥0.25	42	78.6
≥0.50	28	85.7
≥0.75	26	88.5
≥1.0	20	100.0

*Sixteen patients demonstrated no cervical dilatation during this period and only 25% of this group were delivered vaginally.

Comment

The practice of vaginal delivery after previous cesarean section has been a standard of care at this institution for the last 16 years.[10] Lavin et al.[11] reviewed the literature on trial of labor from a 30-year period and concluded that oxytocin use might increase the risk of uterine rupture. Other authors[2,4,6,8,12] and the American College of Obstetricians and Gynecologists have suggested that oxytocin can be used safely without increased maternal or fetal risk. Although the present study was not designed to address safety, we observed no excessive morbidity in our patients, even among women with failed induction or augmentation of labor.

Certain relationships between clinical history and route of delivery among patients undergoing a trial of labor have been confirmed in the present investigation. Although patients with previous arrest of labor may be at increased risk for repeat cesarean section, 50% of our patients with augmentation with this history were delivered vaginally. Hypothetically, if oxytocin was not used, as advocated by Jarrell et al.,[13] most or all of the 64 patients with augmentation would have required abdominal delivery secondary to hypocontractile labor dysfunction. Alternatively, oxytocin use limited to women with nonrecurrent indications, as proposed by Benedetti et al.,[4] would have resulted in 19 more cesarean sections in this series. In reviewing the effect of previous indications on route of delivery, Clark et al.[14] also concluded that exclusion of patients with recurrent conditions was unjustified.

A history of at least one previous vaginal delivery favors a successful trial of labor. Because of standard selection criteria (one previous cesarean section only) and the parity of our patients (median = 1), 72.5% had not experienced a vaginal delivery. Therefore, the recognized advantage of a previous vaginal delivery is lim-

ited to a minority of women and should not be used as a selection criterion for trial of labor or oxytocin use.

Seitchik and Rao[15] analyzed 58 patients with "presumed" recurrent indications (failed progress in labor or cephalopelvic disproportion) who presented for a trial of labor in a subsequent pregnancy at this institution. It was apparent that the original diagnosis was not predictive of repeat abdominal delivery, even in those patients carrying larger infants during the current pregnancy. In our patients with augmentation, although absolute birth weight was significantly different when route of delivery was compared, half of our patients with larger infants in the current pregnancy also were delivered vaginally. It appears that birth weight estimates in excess of birth weight with previous cesarean section should not exclude patients from a trial of labor or use of oxytocin.

Our analysis of cervical dilatation rates before and after oxytocin administration was performed to identify prospectively those patients likely to be delivered vaginally. For patients with augmentation, we selected the first 2 hours of oxytocin treatment for two reasons. Previous work by Seitchik and Castillo[16] noted that most patients with augmentation required less than 4 mU/min to achieve adequate contractility. With this expectation, those patients requiring dose increments each 30 minutes would reach 4 mU by 90 minutes. An additional 30 minutes would then be available to observe the pharmacologic effect of that dosage. Second, the predictive value of this posttreatment observation is limited by how quickly a trend can be detected. For patients with augmentation, any cervical change in the first 2 hours predicted route of delivery in a majority of patients. However, 16 patients without cervical change during this interval still were delivered vaginally. It is apparent that some patients need a period of observation >2 hours before augmentation is considered ineffective. In contrast, patients achieving a minimum of 0.5 cm/hr during these first 2 hours are very likely to be delivered vaginally. This information may be helpful to the clinician attending a trial of labor, during which active-phase arrest occurs and oxytocin is considered.

In summary, oxytocin induction or augmentation during a trial of labor is effective in a majority of patients and is recommended in response to standard obstetric indications. Furthermore, early cervical dilatation during augmented labor has predictive value regarding route of delivery.

REFERENCES

1. ACOG Committee on Obstetrics: maternal-fetal medicine. Statement on vaginal birth after cesarean (VBAC). Washington DC: American College of Obstetricians and Gynecologists, 1985.
2. McGarry JA. The management of patients previously delivered by cesarean section. J Obstet Gynaecol Br Commonw 1969;76:137.
3. Saldana LR, Schulman H, Reuss L. Management of pregnancy after cesarean section. AM J OBSTET GYNECOL 1979;135:555.
4. Benedetti TJ, Platt L, Druzin M. Vaginal delivery after previous cesarean section for a nonrecurrent cause. AM J OBSTET GYNECOL 1982;142:3581.
5. Demianczuk NN, Hunter DJS, Taylor DW. Trial of labor after previous cesarean section: prognostic indicators of outcome. AM J OBSTET GYNECOL 1982;142:640.
6. Flamm BL, Dunnett C. Vaginal delivery following cesarean section: use of oxytocin augmentation and epidural anesthesia with internal tocodynamic and internal fetal monitoring. AM J OBSTET GYNECOL 1984;148:759.
7. Meier PR, Porreco RP. Trial of labor following cesarean section: a two-year experience. AM J OBSTET GYNECOL 1982;144:671.
8. Horenstein JM, Eglinton GS. Oxytocin use during a trial of labor in patients with previous cesarean section. J Reprod Med 1984;29:26.
9. Nie NH, Hull HC, eds. Statistical Package for Social Sciences, X. New York: McGraw-Hill, 1986.
10. Gibbs CE. Planned vaginal delivery following cesarean section. Clin Obstet Gynecol 1980;23:507.
11. Lavin JP, Stephens RJ, Miodomik M, Barden TP. Vaginal delivery in patients with a prior cesarean section. Obstet Gynecol 1982;59:135.
12. Meehan FP, Moolgaoker AS. Vaginal delivery under caudal anesthesia after cesarean section and other major uterine surgery. Br Med J 1972;2:740.
13. Jarrell MA, Ashmeed GG, Mann LI. Vaginal delivery after cesarean section: a five year study. Obstet Gynecol 1985;65:628.
14. Clark SL, Eglinton GS, Beall M, Phelan JP. Effect of indication for previous cesarean section on subsequent delivery outcome in patients undergoing trial of labor. J Reprod Med 1984;29:22.
15. Seitchik J, Rao VRR. Cesarean delivery in nulliparous women for failed oxytocin-augmented labor: route of delivery in subsequent pregnancy. AM J OBSTET GYNECOL 1982;143:393.
16. Seitchik J, Castillo M. Oxytocin augmentation of dysfunctional labor. I. Clinical data. AM J OBSTET GYNECOL 1982;144:899.

The effect of percutaneous estradiol and natural progesterone on postmenopausal bone loss

Bente J. Riis, M.D., Karsten Thomsen, M.D., Verner Strøm, M.D., and Claus Christiansen, M.D.

Glostrup, Denmark

The effect of percutaneous estradiol alone and combined with natural progesterone on postmenopausal bone loss was studied. A total of 57 women who had experienced a natural menopause 6 months to 3 years previously entered the study. After an initial examination the women were allocated in a blinded pattern to treatment with 3 mg of percutaneous estradiol or placebo. The code was broken after 1 year of treatment, and the women receiving estradiol continued with a cyclic addition of progesterone, whereas those receiving placebo continued with placebo. The women were examined every 3 months during the 2 years of treatment. Measurement of the bone mineral content in the forearms (single photon absorptiometry) and the spine and total skeleton (dual photon absorptiometry) showed a significant decrease of 5% to 7% in the placebo group during the 2 years of treatment, whereas it remained constant in all bone compartments in the estradiol group. Addition of progesterone did not influence the results. Biochemical estimates of calcium metabolism changed toward a premenopausal level in the estradiol group but remained unchanged in the placebo group. We conclude that percutaneous estradiol is effective as preventive therapy of postmenopausal bone loss and that addition of progesterone does not influence bone or calcium metabolism. (Am J Obstet Gynecol 1987;156:61-5.)

Key words: Percutaneous estradiol, progesterone, postmenopausal bone loss

After the menopause, women lose bone mineral content at an average rate of 15% to 20% in a 10-year period,[1] which raises the risk that some subjects will manifest osteoporosis later in life. Estrogen replacement therapy has been shown to be the most effective, single modality for the prevention of osteoporosis in women,[2] but it may have some physiologic adverse effects. The metabolic effects of estrogen therapy are influenced by the route of administration. Oral administration is characterized by rapid absorption, a considerable conversion of estradiol to estrone, and a first-pass liver effect, with induction of hepatic protein synthesis. This interaction with liver metabolism may have importance for some of the side effects of estrogen therapy.[3] Percutaneous administration has some advantages because the first-pass liver effect is avoided. Moreover, it has been demonstrated that percutaneous administration of estradiol is effective in ameliorating subjective postmenopausal symptoms.[4] Its effect on bone is, however, unknown.

Combined estrogen-gestagen therapy is an essential feature of postmenopausal hormone replacement therapy.[5] Certain gestagens have, however, been shown to exert adverse effects on lipid metabolism[6] and to influence bone metabolism.[7] Oral administration of micronized progesterone does not adversely affect lipid metabolism.[6] The aim of the present study was to examine the effect of percutaneous estradiol, both alone and in cyclic combination with progesterone, on bone metabolism in early postmenopausal women.

From the Department of Clinical Chemistry, Glostrup Hospital, University of Copenhagen.
Received for publication April 25, 1986; revised July 14, 1986; accepted July 17, 1986.
Reprint requests: Bente Riis, M.D., Department of Clinical Chemistry, Glostrup Hospital, DK-2600 Glostrup, Denmark.

Patients

This study was part of a large double-blind clinical controlled trial carried out at Glostrup Hospital from June 1983 to December 1985. The participants were selected as follows: 9836 questionnaires were sent to women aged 45 to 54 years and 7484 were returned. From information about the last vaginal bleeding, earlier gynecologic operations, and drug intake 558 women fulfilled the following criteria: (1) spontaneous cessation of menstrual bleeding within the preceding 0.5 to 3 years; (2) no treatment with sex hormones or other drugs known to influence calcium metabolism after the menopause. The 558 women were invited to attend a meeting and 397 did attend. After receiving thorough information about the trial 293 gave their written consent to participate (Helsinki Declaration II). The clinical histories were taken and they were given a medical examination including a gynecologic examination with cytologic test, breast palpation, and measurement of resting and supine blood pressures, and blood was sampled. Two hundred seventy women en-

Table I. Clinical data and initial values of bone mass measurements in the two treatment groups (mean ± 1 SEM)

	Hormone group	*Placebo group*
Age (yr)	50.3 ± 0.6	50.5 ± 0.4
Menopausal duration (mo)	15.0 ± 2.2	19.3 ± 1.9
Weight (kg)	61.1 ± 1.8	60.4 ± 1.5
Proximal bone mineral content (U)	38.4 ± 1.1	36.4 ± 1.0
Total body bone mineral content (gm)	3151 ± 126	3011 ± 70
Distal bone mineral content (U)	37.9 ± 1.3	35.7 ± 1.1
Bone mineral density of spine (gm/cm^2)	0.87 ± 0.03	0.87 ± 0.02

tered the trial. All were free of past or present diseases known to influence calcium metabolism or to contraindicate the medications used in the trial. Out of this population 57 women were randomly selected for the present study. After an initial examination the women were allocated to two treatment groups, receiving in a blinded pattern either percutaneous estradiol* (n = 29) or placebo (n = 28) for 1 year. After the first year of treatment, the code was broken; all women receiving 17β-estradiol continued with a supplement of 200 mg of micronized progesterone† from days 13 to 24, and the placebo group continued the placebo cream. The study was thus double blind the first year and single blind the second year.

Trial compliance. Of the 57 women who entered the study 45 women completed all nine examinations (79%). All dropouts took place within the first year of treatment. In the estradiol group nine women left the study: four women because of persistent irregular uterine bleeding; three because of lack of time; one because of aggravation of migraine, and one because of skin irritation at the estradiol application site. In the placebo group two women dropped out because of lack of time and one because of development of hyperthyroidosis. This left 20 patients in the estradiol group and 25 women in the placebo group.

Methods

Bone mineral content of the forearms was measured by single photon absorptiometry, with the use of a Bone Mineral Analyzer 1100 from Mølsgaard Medical (Nuclear Data Corporation). The method estimates bone mineral content in a proximal and a distal region of the forearm with a trabecular bone content of 10% and 45%, respectively (Nilas L, personal communication). The long-term in vivo precision of proximal and distal bone mineral content is 1% and 1.5%.[8] Bone mineral density of the lumbar spine was measured by dual photon absorptiometry on a Lunar Radiation Corporation DP3 scanner. The bone mineral density of the spine was calculated as the bone mineral density values obtained in vertebrae L-2, L-3, and L-4. The precision is 4%.[8] Bone mineral content of the total skeleton was likewise measured by dual photon absorptiometry on a whole-body scanner developed in our laboratory. The precision of the bone mineral content of the total skeleton is 2%.[9]

Intestinal radiolabeled calcium absorption was measured by a modification of the single-isotope technique first described by Marshall and Nordin.[9a] After an overnight fast 10 μCi of calcium chloride Ca 47 was given orally with a carrier dose of 40 mg of calcium ion as calcium chloride and 250 ml of water. Blood samples were taken at 60 and 90 minutes, and the absorbed fraction (the mean values of the results at 60 and 90 minutes) was corrected for lean body mass calculated by the formula of Boddy et al.[9b]

Blood samples were taken and urine was collected in the morning after an overnight fast and tobacco abstinence. Serum estrone and estradiol were measured by radioimmunoassays described elsewhere.[10] Serum alkaline phosphatase was measured enzymatically. Serum calcium was measured by atomic absorptiometry and the serum concentrations were corrected to a serum protein level of 70 gm/L. Fasting urinary hydroxyproline was measured by a spectrophotometric method and corrected for creatinine excretion.

Statistical analysis. For statistical analysis of difference between initial values Student's *t* test for unpaired data was used. To evaluate longitudinal changes in bone mass measurements the initial value was set at 100% and values obtained after institution of treatment were expressed as a percentage of this value. The mean percent change was then used in Student's *t* test for unpaired data.

Results

Table I shows the clinical data and the initial values of bone mass measurements; Table II shows the initial

*Oestrogel cream, 0.6 mg of 17β-estradiol per gram; 28-day cycle: days 1 to 24, 5 gm daily; days 25 to 28, no medication. (Cream applied to the abdominal skin or on the inner side of the thighs or the arm.)

†Utrogestan capsules, 100 mg.

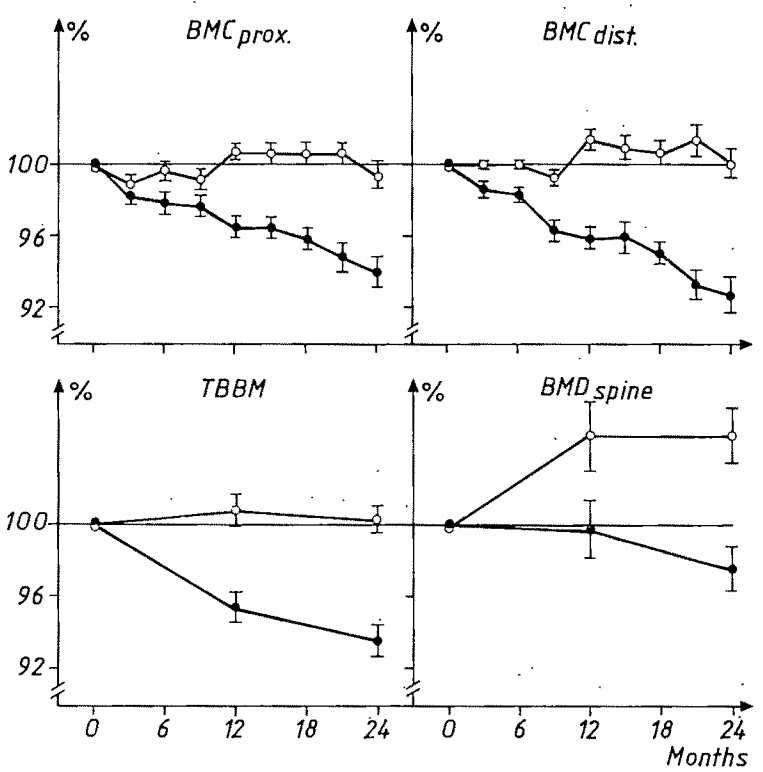


Fig. 1. Mean (± 1 SEM) values of bone mass measurements during the 2 years of treatment. ○ = Hormone group; ● = placebo group. $BMC_{prox.}$ = Proximal bone mineral content; $BMC_{dist.}$ = distal bone mineral content; *TBBM* = total body bone mineral content; BMD_{spine} = bone mineral density of the spine.

values of serum estrogens and biochemical estimates of calcium metabolism. The two treatment groups were comparable.

Fig. 1 gives the mean values of the bone mineral determinations in the four bone compartments. In the placebo group the mean values of all bone mass measurements fell significantly when compared with those in the hormone group, reaching a difference in bone mass of 5% to 7% during 2 years. The addition of progesterone after 1 year of treatment did not influence the results.

Table II shows the changes of serum estrogens, the biochemical estimates of calcium metabolism, and the intestinal radiolabeled calcium absorption. In the hormone group there was a highly significant increase ($p < 0.001$) in serum estrone and estradiol after 3 months of treatment. Thereafter the serum hormone concentrations were virtually unchanged. There was no significant change in serum calcium in either group, whereas both serum alkaline phosphatase and fasting urinary hydroxyproline were decreased in the hormone group compared with the placebo group, reaching significance after 12 months' treatment ($p < 0.01$ to 0.001). Intestinal radiolabeled calcium absorption was unchanged in both groups. None of the variables appeared to be influenced by the addition of progesterone.

Comment

Postmenopausal hormone substitution therapy is widely used, partly to relive climacteric subjective symptoms and partly to prevent postmenopausal bone loss. Percutaneous administration of 17β-estradiol has previously been demonstrated to possess certain advantages over oral estrogen treatment with regard to liver metabolism,[11] and progesterone may have advantages over other gestagens with regard to lipid metabolism.[6]

The present study comprised a representative sample of the Danish population of women who had recently experienced a natural menopause, and the two treatment groups were well matched. We chose a dose of 3 mg of estradiol daily because of its approximate bioequivalence with 2 mg of oral estradiol,[12] which seems to be optimal in terms of preventing bone loss.[13] Studies have shown, furthermore, that a single daily dose of 2 or 4 mg of percutaneous estradiol results in serum estradiol levels that are maintained for 48 hours and that are comparable to serum levels after oral administra-

Table II. Serum estrogens and biochemical estimates of calcium metabolism before and during treatment (mean ± 1 SEM)

	Hormone group			
	Initial value	*3 mo*	*12 mo*	*24 mo*
Serum estrone (pg/ml)	68 ± 5	141* ± 16	215* ± 45	204* ± 32
Serum estradiol (pg/ml)	34 ± 18	180* ± 32	218* ± 62	200* ± 34
Serum calcium (mmol/L)	2.49 ± 0.02	2.44 ± 0.01	2.45 ± 0.01	2.47 ± 0.02
Serum alkaline phosphatase (U/L)	166 ± 7	161 ± 10	127* ± 6	123* ± 9
Fasting urinary hydroxyline (mmol/mol)	14.0 ± 0.7	12.5 ± 0.9	9.3† ± 0.4	10.2† ± 0.7
Intestinal radiolabeled calcium absorption (U)	24.4 ± 1.6	—	24.5 ± 1.3	24.1 ± 1.3

*Change in hormone group versus placebo group: $p < 0.001$.
†Change in hormone group versus placebo group: $p < 0.01$.

tion.[14] The dispensing form involves only minor problems: At the beginning of the study most of the women felt it "strange" but thereafter it was well accepted; one woman experienced itching at the application site, without any visible cutaneous sign.

The biochemical calcium metabolic variables changed during treatment in the same way as with oral estrogen treatment,[2] that is, a decrease to the premenopausal level of bone turnover rate.

The methods used for measuring bone mass have a precision that makes them suitable for longitudinal studies. Percutaneous estrogen treatment kept bone mass constant, whereas placebo treatment resulted in a significant loss of bone mineral content in all bone compartments. The mean differences between changes in the two groups, measured either in the total skeleton or locally, were virtually the same after 2 years of treatment. Which bone site (or type of measurement) is the most optimal for studies in osteoporosis has been the subject of heated discussion,[15, 16] but the present study clearly demonstrates that all four measurement systems were able to show the difference between the two groups. It should be noted, however, that the standard error of the mean was lowest for the proximal bone mineral content (0.7% to 0.8% at 24 months) and highest for the bone mineral density of the spine (1.5% to 1.7% at 24 months). This indicates that study of more patients is required to demonstrate the reponse to estrogen treatment if the spine scanner is used than if the forearm scanner is used.

Unopposed oral or percutaneous estrogen treatment for longer periods increases the risk of endometrial cancer, whereas this risk is abolished when a cyclic gestagen component is added.[5] Gestagen also prevents irregular bleeding. In the present study irregular uterine bleeding was observed in the hormone group when treated with estrogen alone, but they had regular bleeding in the second year of treatment. It is also very important that the chosen gestagen exert little or no adverse effects on lipid metabolism; progesterone seems to be such an agent.[6] We conclude that treatment with percutaneous estradiol is a possible alternative to oral administration in terms of preventing postmenopausal bone loss and that addition of progesterone does not influence bone or calcium metabolism.

We thank Laboratoires Besins Iscovesco for supplying Oestrogel cream, Utrogestan capsules, and placebo.

REFERENCES

1. Smith DM, Khairi MRA, Norton J, Johnston CC Jr. Age and activity effects on rate of bone mineral loss. J Clin Invest 1976;58:716-21.
2. Christiansen D, Christensen MS, McNair P, Hagen C, Stocklund KE, Transbøl I. Prevention of early postmenopausal bone loss: controlled 2-year study in 315 normal females. Eur J Clin Invest 1980;10:273-9.
3. Geola FL, Frumar AM, Tataryn IV, et al. Biological effects of various doses of conjugated equine estrogens in postmenopausal women. J Clin Endocrinol Metab 1980; 51:620-5.
4. Brincat M, De Trafford JC, Lafferty K, Studd JWW. Peripheral vasomotor control and menopausal flushing—a preliminary report. Br J Obstet Gynaecol 1984;91: 1107-10.
5. Gambrell RD, Massey FM, Castaneda TA, Ugenas AJ, Ricci CA. Reduced incidence of endometrial cancer among postmenopausal women treated with progestogens. J Am Geriatr Soc 1979;27:389-94.
6. Ottosson UB. Oral progesterone and estrogen/progestogen therapy. Effects of natural and synthetic hormones on subfractions of HDL cholesterol and liver proteins. Acta Obstet Gynecol Scand 1984;127(suppl):5-37.
7. Christiansen C, Nilas L, Riis BJ, Rødbro P. Uncoupling of bone formation and resorption by combined oestrogen and progestagen therapy in postmenopausal osteoporosis. Lancet 1985;2:800-1.
8. Nilas L, Borg J, Gotfredsen A, Christiansen C. Comparison of single and dual photon absorptiometry in postmenopausal bone mineral loss. J Nucl Med 1985;218: 329-33.
9. Gotfredsen A, Borg J, Christiansen C, Mazess RB. Total body bone mineral in vivo by dual photon absorptiometry. I. Measurement procedures. Clin Physiol 1984;4:343-55.
9a. Marshall DH, Nordin BEC. Kinetic analysis of plasma radioactivity of oral ingestion of radiocalcium. Nature 1969;222:797-9.
9b. Boddy K, King PC, Thume R, Weyers E. The relation

Placebo group			
Initial value	*3 mo*	*12 mo*	*24 mo*
66 ± 4	58 ± 7	65 ± 5	65 ± 4
11 ± 2	14 ± 6	7 ± 1	7 ± 1
2.49 ± 0.01	2.49 ± 0.02	2.46 ± 0.02	2.48 ± 0.02
173 ± 8	171 ± 8	173 ± 8	177 ± 9
15.4 ± 0.7	14.8 ± 1.0	12.6 ± 0.7	15.4 ± 1.3
25.5 ± 1.6	—	24.7 ± 1.2	25.6 ± 1.0

of total body potassium to height, weight, and age in normal adults. J Clin Pathol 1972;25:512-7.
10. Jensen J, Riis BJ, Hummer L, Christiansen C, Rødbro P. The effects of age and body composition on circulating serum oestrogens and androstenedione after the menopause. Br J Obstet Gynaecol 1985;92:260-5.
11. Holst J. Percutaneous estrogen therapy. Endometrial response and metabolic effects. Acta Obstet Gynecol Scand 1983;115(suppl):7.
12. Fåhraeus L, Larsson-Cohn U. Oestrogens, gonadotrophins and SHBG during oral and cutaneous administration of oestradiol 17β to menopausal women. Acta Endocrinol 1982;101:592-6.
13. Christensen MS, Hagen C, Christiansen C, Transbøl I. Dose-response evaluation of cyclic estrogen/gestagen in postmenopausal women: placebo-controlled trial of its gynecologic and metabolic actions. Am J Obstet Gynecol 1982;144:873-9.
14. Lyrenäs S, Carlström K, Bäckström T, von Schoultz B. A comparison of serum oestrogen levels after percutaneous and oral administration of oestradiol 17β. Br J Obstet Gynaecol 1981;88:181-7.
15. Riggs BL, Wahner HW, Duun WL, Mazess RB, Offord KP, Melton LJ III. Differential changes in bone mineral density of the appendicular and axial skeleton with aging. J Clin Invest 1981;67:328-35.
16. Mazess RB, Peppler WW, Chesney RW, Lange TA, Lindgren U, Smith E. Does bone measurement on the radius indicate skeletal status? Concise communication. J Nucl Med 1984;25:281-8.

Long-term effects of percutaneous estrogens and oral progesterone on serum lipoproteins in postmenopausal women

Jytte Jensen, M.D., Bente J. Riis, M.D., Verner Strøm, M.D., Lisbeth Nilas, M.D., and Claus Christiansen, M.D.

Glostrup, Denmark

Serum lipids and lipoproteins were examined in a group of 45 healthy postmenopausal women who were treated for 2 years with either 3 mg of percutaneous estradiol (n = 20) or placebo (n = 25). Percutaneous estradiol was given alone during the first year of treatment and in combination with oral micronized progesterone (200 mg) for 12 days of each cycle during the second year. The women were examined every 3 months throughout the 2 years. Percutaneous estrogen therapy significantly reduced total serum cholesterol and low-density lipoprotein cholesterol, whereas no significant differences were observed in serum triglycerides and high-density lipoprotein cholesterol. Addition of oral progesterone during the second year of treatment did not produce any significant alterations in serum total cholesterol or low-density lipoprotein cholesterol, both of which remained significantly reduced. Serum triglycerices remained virtually unchanged, whereas a slight but significant increase ($p < 0.05$) was observed in high-density lipoprotein cholesterol levels at the end of the study period. We conclude that percutaneous estrogen administration produces changes in total serum cholesterol and low-density lipoprotein cholesterol levels similar to those observed after oral estrogen administration. However, the magnitude and time course of the response seem to be modulated by the route of administration. Addition of ora micronized progesterone does not influence the beneficial estrogenic actions on serum lipids and lipoproteins and seems to be a proper "progestogen" in percutaneous estrogen therapy. (Am J Obstet Gynecol 1987;156:66-71.)

Key words: Percutaneous estrogens, serum lipoproteins, postmenopause, oral progesterone

Postmenopausal estrogen therapy is widely used for the treatment of climacteric complaints, and the recognized efficacy of estrogens in preventing postmenopausal bone loss might result in a future rise in the number of women receiving such therapy.

Some metabolic side effects of estrogen therapy seem to be caused by alteration of hepatic functions. Thus changes in lipid and protein metabolism following administration of natural estrogens are believed to be more a consequence of the oral route of administration than of the estrogen treatment per se. Percutaneous administration has the initial advantage of bypassing the portal circulation, and this route of administration has been shown to induce less alteration in the hepatic synthesis of certain lipids[1] and proteins[2] than oral administration. Moreover, physiologic levels of serum estradiol and estrone comparable to the premenopausal range can be attained with percutaneous estrogen administration,[1-3] presumably because percutaneous absorption is slow[3] and the gastrointestinal interconversion of estrogen metabolites is avoided. The effect of orally administered estrogens on serum lipoproteins might be considered advantageous from a cardiovascular point of view, since the therapy increases the levels of high-density lipoprotein (HDL) cholesterol and decreases the levels of low-density lipoprotein (LDL) cholesterol.[4] However, cyclic estrogen/progestogen treatment is recommended, because the addition of progestogen reduces the risk of endometrial hyperplasia[5] and cancer.[6] Unfortunately, some progestogens have been found to reduce[7] or even reverse[8] the beneficial actions of estrogen on serum lipoproteins, although this depends on the type and dose of the implicated progestogen. Recently, "natural" micronized progesterone has been reported to have no adverse effects on lipoprotein metabolism.[8]

Percutaneous estrogen administration has proved to be effective in alleviating climacteric complaints,[2] but the long-term effects on serum lipids and lipoproteins have not been examined in controlled trials, nor has the long-term effect of percutaneous estrogen opposed by oral progesterone on lipoproteins been studied.

In the present placebo controlled study of 45 postmenopausal women, we report on the changes in lipoprotein profiles during 2 years of percutaneous

From the Departments of Clinical Chemistry and Gynaecology and Obstetrics, Glostrup Hospital, University of Copenhagen.
Received for publication April 15, 1986; revised July 14, 1986; accepted July 17, 1986.
Reprint requests: Jytte Jensen, Department of Clinical Chemistry, Glostrup Hospital, University of Copenhagen, DK-2600 Glostrup, Denmark.

Table I. Initial values and clinical data in the two groups of postmenopausal women

Group	*n*	*Age (yr)*	*Menopausal age (mo)*	*Weight (kg)*	*Serum triglyceride cholesterol (mmol/L)*	*Serum cholesterol (mmol/L)*	*Serum HDL cholesterol (mmol/L)*	*Serum LDL cholesterol (mmol/L)*
Percutaneous estradiol	20	50.3 ± 2.7	15 ± 10	61 ± 8	0.9 ± 0.3	6.7 ± 1.2	1.8 ± 0.4	4.4 ± 1.1
Placebo	25	50.1 ± 1.9	19 ± 9	60 ± 8	1.1 ± 0.5	6.2 ± 1.2	1.7 ± 0.4	4.0 ± 1.2

Values are given as mean ± 1 SD.

estrogen therapy opposed for 1 year by oral progesterone.

Material and methods

Participants. This study was part of a large double-blind controlled trial. The participants were selected as follows: of 9836 questionnaires sent to all women aged 45 to 54 years in Copenhagen County, 7484 (76%) were returned. From information about the date of the last vaginal bleeding, earlier gynecologic operations, and drug intake, 558 women fulfilled the following criteria: (1) spontaneous cessation of menstrual bleeding within the preceding 6 months to 3 years; (2) no treatment with sex hormones or drugs known to influence calcium metabolism after the menopause. The 558 women were invited to an information meeting, which 397 attended. After receiving thorough information about the trial, 293 gave their written consent to participate (Helsinki Declaration II). A medical examination was performed, which included a clinical history, gynecologic examination with a cytologic test, breast palpation, and blood sampling. Of the 270 women who entered the trial, all were free of past or present diseases known to influence calcium metabolism or to contraindicate the medications of the trial. Of this population 57 women were randomly selected for the present 2-year study. After three initial examinations the women were allocated to two treatment groups, receiving either cyclic percutaneous estradiol* (n = 29) or placebo (n = 28) in a 28-day cycle. After 1 year of treatment the code was broken and the participants in the estradiol group received oral micronized progesterone† (200 mg) for 12 days (days 13 to 24) of the treatment cycle in addition to the percutaneous estradiol, whereas the placebo group continued to receive the same medication. Hence the study was double-blind throughout the first year and single-blind the second year. No dietary instructions were given. During both treatment years all participants were examined every three months (nine examinations in all). The trial was approved by the Ethical Committee of Copenhagen County.

Trial compliance. Fifty-seven women entered the study, and 45 women completed all examinations (79%). In the estradiol group 9 women left the study, all within the first year. Four women dropped out because of persistent irregular bleedings, three women because of lack of time, one because of aggravation of migraine, and one because of skin irritation at the application site of the estradiol gel (without any visible reaction). Three women in the placebo group dropped out, two because of lack of time and one because thyrotoxicosis developed. The estradiol group finally consisted of 20 women and the placebo group 25 women.

Table I gives the clinical data and initial values of serum lipids and lipoproteins in the two groups of women who completed the study. The groups were comparable.

Methods. Blood samples were drawn in the morning after an overnight fast and tobacco abstinence between days 21 and 24 of the tablet cycle.

Serum lipids and lipoproteins. The HDL lipoprotein fraction was separated from chylomicrons, LDL, and very low-density lipoprotein (VLDL) by the Mg^{2+}/dextran sulphate precipitation technique. Total serum cholesterol and HDL cholesterol were determined by an enzymatic technique with use of an LKB Ultrolab system. The intra-assay and interassay variations were 1% and 2% (total cholesterol) and 2% and 5% (HDL cholesterol), respectively. Serum triglycerides were determined by an enzymatic technique with use of an LKB 8600 reaction rate analyzer. The intra-assay and interassay variations of this method are 5% and 8%.

LDL cholesterol was then estimated according to the procedure of Friedewald et al.[9] The intra-assay and interassay variations of this estimation were 5% and 10%.

Serum estradiol was measured with use of antiserum supplied by Milab, and standards were prepared with use of 17β-estradiol supplied by Merck. The intra-assay and interassay variations were 6% and 15%, and the sensitivity 15 pmol/L.

Serum estrone was measured with use of antiserum supplied by Statens Seruminstitut, and standards were

*Oestrogel: cream, 60 mg of estradiol/100 gm. Days 1 to 24: 5 gm daily, applied to the skin of the abdomen or on the inside of the arms or the thighs.

†Utrogestan: capsules, 100 mg.

Table II. Bleeding pattern during treatment with percutaneous estrogen before and after addition of oral progesterone

Percutaneous estradiol (first year)				*Percutanaeous estradiol plus oral progesterone (second year)*			
Bleeding pattern	*n*	*Curettage (no. of patients)*	*Endometrial histology*	*Bleeding pattern*	*n*	*Curettage (no. of patients)*	*Endometrial histology*
None	12	–	–	Regular	7	–	–
				None	5	–	–
Regular	2	–	–	Regular	2	–	–
				Irregular	0	–	–
Irregular	6	2	Estrogenic	Regular	5	–	–
				Irregular	1	1	Estrogenic

prepared with use of estrone supplied by Sigma. The intra-assay and interassay variations were 11% and 11%, and the sensitivity 100 pmol/L.

Statistical analysis. For each participant the pretreatment value was assessed 100%, and the subsequent measurements were expressed as a percentage of this value. The cumulated therapeutic response was calculated in each participant as the mean of the four measurements during the first and second year of treatment, respectively.

Student's *t* test for paired data was used to test the significance of difference *within* the groups, and Student's *t* test for unpaired data was used *between* the groups.

Results

Uterine bleeding. Table II shows the bleeding pattern in the group receiving percutaneous estradiol before and after addition of oral progesterone. During the year of estradiol monotherapy, eight women (40%) experienced withdrawal bleeding which, however, only occurred regularly in two women (10%). After addition of progesterone, 14 women (70%) experienced regular withdrawal bleeding, whereas only one woman had irregular bleeding.

Three women with irregular bleeding underwent curettage and the endometrial histologic finding was estrogenic in all (Table II). None of the women in the placebo group experienced uterine bleeding.

Body weight. Body weight remained virtually unchanged in the hormone group throughout the 2 years of the study (61.1 ± 8.0 kg initially versus 61.5 ± 8.8 kg in first year versus 61.8 ± 9.3 kg in second year; NS), whereas a significant increase was observed in the placebo group after 1 and 2 years, respectively (60.4 ± 7.5 kg versus 61.1 ± 7.6 kg in first year, $p < 0.005$, and 61.6 ± 8.4 kg in second year, $p < 0.01$).

Serum estradiol and estrone. Fig. 1 shows the changes in serum estradiol and estrone in the two treatment groups at 3, 6, 12, and 24 months of treatment.

Both estrogens were significantly increased in the hormone group at 3 months of treatment ($p < 0.001$). The serum concentrations of both estrogens, however, continued to increase, reaching maximum levels only after 6 months of therapy. This pattern was most pronounced for serum estrone. After 6 months of treatment the serum levels of both estrogens remained virtually constant throughout the rest of the study. The initial estrone/estradiol ratio was >2, but after 6 months of percutaneous estrogen treatment, the ratio was reduced to 1 and this ratio was maintained for the rest of the study.

In the placebo group both estrogens remained within the postmenopausal range throughout the study period.

Serum lipids and lipoproteins. Fig. 2 compares the effect of treatment with cutaneous estradiol, before and after addition of oral progesterone, on serum lipids and lipoproteins with that of placebo. During the first year of cutaneous estradiol therapy the total serum levels of cholesterol and LDL cholesterol were significantly reduced compared to placebo, whereas no significant differences were observed in the serum levels of triglycerides and HDL cholesterol.

Addition of oral progesterone during the second year of treatment did not produce significant alterations in serum total cholesterol and LDL cholesterol, both of which remained significantly reduced in the hormone group. Serum triglycerides remained virtually unchanged during the second year, and no significant differences were observed between the two groups. Throughout the first 6 months after addition of progesterone, HDL cholesterol levels were similar in the two groups, whereas the HDL levels tended to increase during the last 6 months of combined hormone therapy. This increase was significant ($p < 0.05$) at the end of the study period.

Fig. 3 compares the cumulated treatment responses on serum lipids and lipoproteins (that is, mean of four examinations during the first and second year, respectively) in the two groups. The first year of cutaneous estradiol therapy resulted in significantly reduced levels of total cholesterol and LDL cholesterol, both compared to the corresponding initial values ($p < 0.05$, re-

spectively) and compared to placebo ($p < 0.001$, respectively). Serum concentration of triglycerides and HDL cholesterol were significantly increased in the hormone group compared to the placebo group ($p < 0.001$, respectively); however, the changes were not significantly different from initial values. During the second year of treatment, when oral progesterone was added to the percutaneous estradiol, the cumulated response resulted in significant and slightly greater reductions in total cholesterol ($p < 0.01$) and LDL cholesterol ($p < 0.001$) compared to those after percutaneous estradiol alone. However, the differences between the cumulated responses after the first and the second year were not significant. In contrast, no significant changes were observed in serum triglycerides and HDL cholesterol during the second year, although HDL cholesterol levels remained higher in the hormone group. The difference between the cumulated response in HDL after the first and the second year was not significant.

Correlations between the cumulated response of estradiol (that is, mean of four examinations during treatment) and the cumulated change in LDL and total cholesterol (mean of eight examinations), respectively, were significant: estradiol versus LDL, $r = -0.69$, $p < 0.001$, estradiol versus total cholesterol, $r = -0.63$, $p < 0.01$.

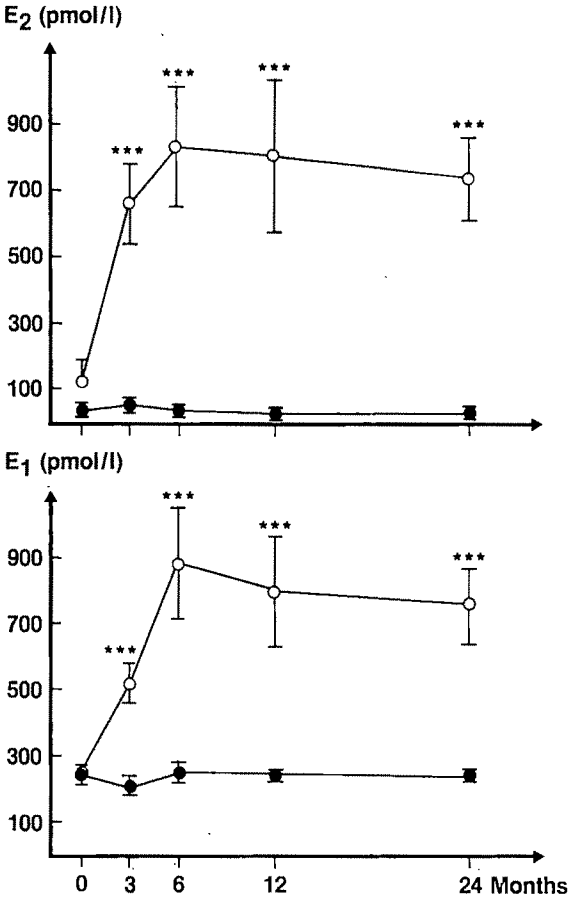


Fig. 1. Serum estradiol *(E_2)* and estrone *(E_1)* during 2 years of treatment with percutaneous estradiol (○) and placebo (●). Values are given as mean ± 1 SEM. Significance of difference: *** $p < 0.001$.

Comment

Both natural and surgical menopause are accompanied by adverse changes in serum lipids and lipoproteins, which are associated with an increased risk of developing cardiovascular diseases.[10] These adverse changes are generally considered a consequence of the ceased ovarian estrogen production, since treatment with oral estrogens reverses the negative trends in lipoprotein profiles by lowering LDL cholesterol and increasing HDL cholesterol.[4] Hence postmenopausal estrogen therapy might be considered advantageous from a cardiovascular point of view, and epidemiologic studies have suggested that postmenopausal estrogen replacement therapy has an overall protective effect against death from ischemic heart disease.[11] In the present study percutaneous estrogen therapy significantly reduced the serum levels of total cholesterol and LDL cholesterol. HDL cholesterol levels tended to be higher after percutaneous estrogens than after placebo, and the cumulated treatment response resulted in a small but significant rise in HDL cholesterol levels. Previous studies of percutaneous estrogen therapy have reported only minor and insignificant changes in serum lipoproteins,[1, 12] despite significant increases in serum estradiol and estrone. These were short-term studies, however, and did not exceed 6 months of therapy. The short duration of treatment probably explains the different findings, since in the present study significant changes in lipids and lipoproteins were only observed after 6 months of treatment. Moreover, the serum levels of both estrogens continued to increase during the first 6 months of percutaneous treatment and only reached the maximum and plateau levels after this period. Furthermore, significant correlations were demonstrated between the cumulated response of estradiol and the cumulated change in LDL and total cholesterol, respectively. Changes in lipoprotein profiles after oral estrogen use have previously been reported to be dose related,[7] and the present response after use of 3 mg of percutaneous estradiol corresponds to the results obtained with 1 mg of oral 17β-estradiol. However, unlike the present study the changes in lipoprotein profiles during oral therapy occurred almost immediately.[7] A possible explanation for the different time/effect course of oral and percutaneous administration of estrogen on lipoproteins might be the route of administration. By the oral route, estrogens are rapidly absorbed with peak plasma concentrations within the first

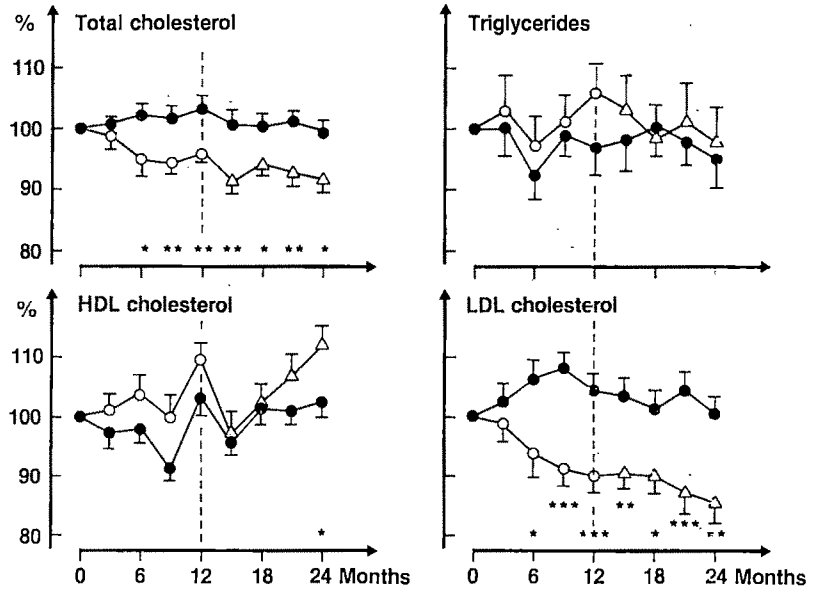


Fig. 2. Serum lipids and lipoproteins during treatment with percutaneous estrogens (○), percutaneous estrogens combined with cyclic progesterone (△), or placebo (●). Values are given as mean ± 1 SEM and expressed in percent of initial values. Significance of difference from the placebo group: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

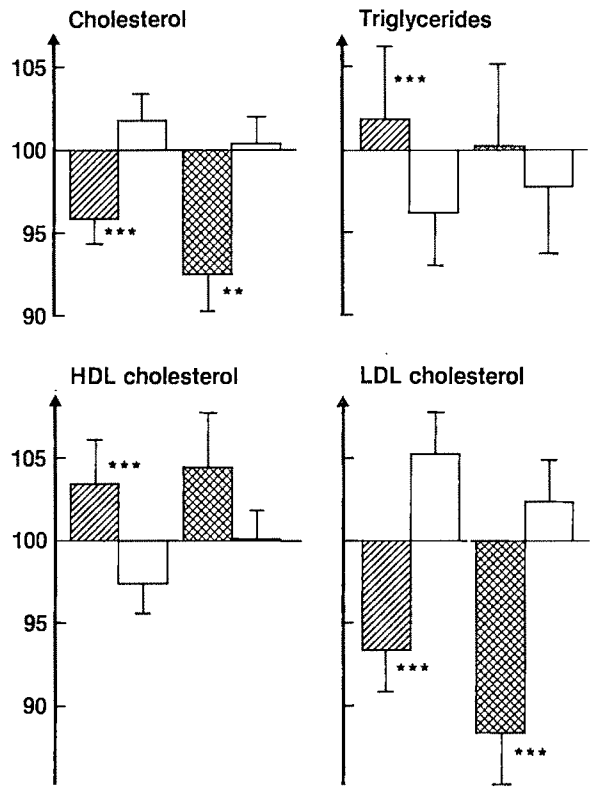


Fig. 3. Cumulated treatment response on serum lipids and lipoproteins following percutaneous estrogens *(striped bar)*; percutaneous estrogens combined with cyclic progesterone *(hatched bar)*; and placebo *(open bar)*. Values are given as mean ± 1 SEM and expressed in percent of initial values. Significance of difference from the placebo group: ** $p < 0.01$, *** $p < 0.001$.

hours of administration,[13] and a large part of the estradiol is converted to estrone in the gastrointestinal tract.[13] Binding of estrone in mouse liver has been shown to be greater than that of estradiol,[14] and estrone has been found to be twice as active as estradiol on rat hepatocytes.[15] Therefore it might be hypothesized that the high estrone levels, which are a consequence of hepatic extraction after oral administration, might result in a greater stimulation of the liver. This would induce changes in lipoprotein metabolism of greater magnitude and with a shorter time course than similar doses of percutaneous estrogens, in which absorption is slow and initially bypasses the liver and in which the estradiol/estrone ratio remains within the premenopausal range.

Cyclic addition of a progestogen is recommended for postmenopausal estrogen replacement therapy, since it reduces the risk of endometrial hyperplasia[5] and neoplasia.[6] Unfortunately, some progestogens have been found to affect serum lipids adversely,[8] although these actions depend on the type and dose of the implicated progestogen, as well as on the concomitant use of estrogen.[8] In the present study, addition of 200 mg of micronized progesterone did not alter the estrogenic effect on serum lipoproteins. However, our study design does not allow us to determine whether the changes in serum lipids during the second year of treatment are caused by the continuous administration of estradiol or by the addition of progesterone. However, the findings agree with previous studies on micronized progesterone,[8] indicating that progesterone does not

influence or at least does not adversely affect lipoprotein metabolism. The dose of 200 mg of progesterone induced regular withdrawal bleedings in 70% of the hormone-treated women compared to 10% during the year with percutaneous estrogens alone. Twelve women did not experience bleeding during the estrogen monotherapy, and in five women, bleeding did not occur after the addition of progesterone. A recent study reported a similar frequency of bleeding, and 200 mg of micronized progesterone taken orally for 10 days was found to induce significant biochemical and morphologic effects on the endometrium.[16] These findings, plus the fact that progesterone was administered for 12 days in each cycle during the present study, suggest that adequate protection of the endometrium was obtained during the combined therapy.

We conclude that percutaneous administration of estrogen produces changes in total serum cholesterol and LDL cholesterol similar to those observed with oral estrogens. However, the magnitude and time course of the response seem to be modulated by the route of administration. Addition of oral micronized progesterone does not influence the beneficial actions of estrogen on serum lipids and lipoproteins and seems to be a proper "progestogen" in percutaneous estrogen therapy.

We wish to thank Laboratories Besins Iscovesco for supplying the Oestrogel cream, Utrogestan capsules, and placebo.

REFERENCES

1. Basdevant A, De Lignieres B, Guy-Grand B. Differential lipemic and hormonal responses to oral and parenteral 17β-estradiol in postmenopausal women. Am J Obstet Gynecol 1983;147:77-81.
2. Fåhraeus L, Larsson-Cohn U. Oestrogens, gonadotrophins and SHBG during oral and cutaneous administration of oestradiol-17β to menopausal women. Acta Endocrinol 1982;101:592-6.
3. Whitehead MI, Townsend PT, Kitchen Y, et al. Plasma steroid and protein hormone profiles in postmenopausal women following topical administration of oestradiol 17β. In: Mauvais-Jarvis P, Vickers CFH, Wepierre J, eds. Percutaneous absorption of steroids. London: Academic Press, 1980:231-48.
4. Wallace RB, Hoover J, Barrett-Connor E, et al. Altered plasma lipid and lipoprotein levels associated with oral contraceptive and estrogen use. Lancet 1979;2:111-4.
5. Whitehead MI, King RJB, McQueen J, Campbell S. Endometrial histology and biochemistry in climacteric women during estrogen and oestrogen/progestogen therapy. J R Soc Med 1979;72:322-7.
6. Hammond CB, Jelovsek KR, Lee KL, Creasman WT, Parker RT. Effects of long-term estrogen replacement therapy. II. Neoplasia. Am J Obstet Gynecol 1972;123: 537-47.
7. Jensen J, Nilas L, Christiansen C. Cyclic changes in serum cholesterol and lipoproteins following different doses of combined postmenopausal hormone replacement therapy. Br J Obstet Gynaecol 1986;93:613-8.
8. Fåhraeus L, Larsson-Cohn U, Wallentin L. L-Norgestrel and progesterone have different influences on plasma lipoproteins. Eur J Clin Invest 1983;13:447-53.
9. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.
10. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. Am J Med 1977;62: 707-14.
11. Ross RK, Paganini-Hill A, Mack TM, Arthur M, Henderson BE. Menopausal estrogen therapy and protection from death from ischaemic heart disease. Lancet 1981; 1:858-60.
12. Fåhraeus L, Larsson-Cohn U, Wallentin L. Lipoproteins during oral and cutaneous administration of oestradiol-17β to menopausal women. Acta Endocrinol 1982;101: 597-602.
13. Yen SSC, Martin PL, Burnier AM, Czekala NM, Greaney MO Jr, Callentine MR. Circulating estradiol, estrone and gonadotropin levels following the administration of orally active 17β-estradiol in postmenopausal women. J Clin Endocrinol Metab 1975;40:518-21.
14. Stone GM, Baggett B, Donelly RB. The uptake of triated oestrogens by various organs of the ovariectomized mouse following intravenous administration. J Endocrinol 1963; 27:271-80.
15. Gallagher TF, Mueller MN, Kappas A. Estrogen pharmacology. IV. Studies on the structural basis for estrogen-induced impairment of liver functions. Medicine 1966; 45:471-9.
16. Lane G, Siddle NC, Ryder TA, Pryse-Davies J, King RJB, Whitehead MI. Dose dependent effects of oral progesterone on the oestrogenised postmenopausal endometrium. Br Med J 1983;287:1241-5.

Use of the urinary director appliance for management of voiding problems after radical vulvectomy

Karen L. Ilika, M.D., and Patrick Duff, M.D.
Tacoma, Washington

This case report describes the use of a simple appliance for the control of the urinary stream in four patients who experienced difficulty voiding after radical vulvar surgical procedures. (Am J Obstet Gynecol 1987;156:72-3.)

Key words: Vulvectomy, urinary stream, funnel

Certain patients may experience voiding problems after radical genitourinary surgical procedures. We recently evaluated four women who complained of persistent deviation of the urinary stream after radical vulvectomy. The resulting problem was that of urine spraying out between the top of the toilet bowl and the toilet seat. To our knowledge, this phenomenon has not been previously described.

Methods

The Sani-Fem Urinary Director (Sani-Fem, Downey, California) is sold as an aid for women who backpack. Its purpose is to allow women to urinate in the standing position without disrobing. The appliance is an elliptically shaped plastic funnel measuring approximately 5 inches in its longest diameter. The elliptical opening is held against the perineum. An outflow spout located at one end of the ellipse directs the urine stream away from the user (Fig. 1). We offered this device to four patients who complained of uncontrolled spraying of urine when voiding after radical vulvar surgical procedures.

Results

The first patient to try this device was a 63-year-old woman who had undergone a radical vulvectomy for malignant melanoma 18 months before our evaluation. She underwent an anterior repair 6 months after vulvectomy because of symptoms of stress urinary incontinence. At the time of evaluation she was again having incontinence symptoms as well as difficulty in directing the urine stream. She used the funnel device while awaiting further operation. After experimenting with various positions, she found that the device worked best for her in the sitting position with the spout directed posteriorly. She subsequently underwent a suprapubic urethropexy and scar revision of the perineum, resulting in improvement of her symptoms.

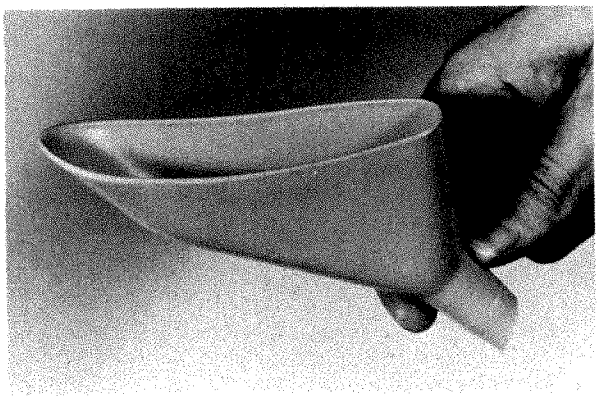


Fig. 1. The Sani-Fem Urinary Director.

Our second patient was a 61-year-old woman who began to spray urine after a radical vulvectomy and radiation therapy for squamous cell carcinoma. She described her difficulty as being intermittent and used the funnel most often when away from home. She was quite satisfied with this solution and had been using it for 18 months.

Our third and fourth patients underwent radical vulvectomy for Stage II squamous cell carcinoma of the vulva. Both patients developed the problem of spraying urine in the immediate postoperative period. One used the urinary director successfully while still in the hospital. She subsequently has had less need for it. The other patient, a 51-year-old businesswoman, tried the device and found that it contained the spray of urine well. However, she discovered that a disposable plastic bag over her hand provided an inexpensive and convenient shield that served the same purpose. For her lifestyle this innovative alternative proved to be more acceptable.

From the Department of Obstetrics and Gynecology, Madigan Army Medical Center.
The opinions expressed herein are those of the authors and are not to be construed as those of the Army Medical Department.
Received for publication April 10, 1986; accepted June 24, 1986.
Reprints will not be available.

Comment

Although not previously described, the problem of postoperative spraying of urine is probably quite common after radical vulvar operation. The onset of this problem may be in the immediate postoperative period or it may be delayed. It causes a great deal of distress for certain patients. Physicians need to be aware of and inquire about its occurrence. The Sani-Fem Urinary Director is an inexpensive (approximately $9) and easily used device that avoids the need for internal instrumentation and the resultant risk of infection. In selected patients, it presents an attractive alternative to further operation. Patients who may benefit from its use should be made aware of its availability.

Liposarcoma presenting on the vulva

John J. Brooks, M.D.,* and Virginia A. LiVolsi, M.D.
Philadelphia, Pennsylvania

A myxoid liposarcoma of the vulvar perineum in a 15-year-old girl is described. The tumor recurred as a poorly differentiated round cell liposarcoma and resulted in the patient's death. Clinicians and pathologists should be aware that this tumor may rarely present on the vulva. (Am J Obstet Gynecol 1987;156:73-5.)

Key words: Vulva, sarcomas, liposarcoma

During an analysis of all of our vulvar soft tissue tumors, we encountered an exceptional case that was not originally recognized. A myxoid liposarcoma presenting on the vulva is reported. Wide local excision and postoperative radiotherapy may have been curative in this initially low-grade sarcoma. Since this lesion may mimic other soft tissue tumors, it is important that its rare occurrence on the vulva be recognized.

Case report

A 15-year-old white girl complained of a large mass in the groin, in December 1975. Physical examination disclosed a 6.5 cm protrusion on the vulvar perineum. Encroachment on the posterior vagina and anterior portion of the rectum was noted on internal examination. The initial clinical diagnosis was soft tissue sarcoma. An 18 cm tumor was completely removed at operation through an abdominoperineal approach. At first, the lesion was misdiagnosed as a cellular neurofibroma, and no further therapy was given.

Twenty months later, in August 1977, the tumor recurred as an 11 cm mass in the adjacent tissues of the left posterior medial thigh. At this time the initial tissue was reviewed and the diagnosis changed to a low-grade myxoid liposarcoma. Small, widely scattered cells were present in a myxoid-ground substance with an accompanying capillary network (Fig. 1). Only a rare mitotic figure was noted and the tumor contained no evidence of necrosis or pleomorphism. In contrast, the recurrence was a high-grade sarcoma with abundant mitoses, marked cellularity, necrosis, and nuclear pleomorphism with tumor giant cells (Fig. 2).

After the recurrence, the patient was given chemotherapy to no avail. The patient died of disease 31 months after presentation, of local disease and renal failure. There was no clinical evidence of metastatic disease at the time of death. No autopsy was performed.

Comment

Liposarcoma is one of the most common soft tissue sarcomas of adults. The majority of cases occur on the lower extremities, particularly the thigh; the retroperitoneum is also a frequent location. Although liposarcoma has been reported in unusual locations, there appear to be no reports of its presentation on the vulva, nor are there vulvar cases contained within larger series on liposarcomas. The only previous reference to this topic is that by Gompel and Silverberg,[1] who referred to a single case of liposarcoma occurring on the vulva; no clinical or pathologic details were provided.

It is debatable whether the tumor described here arose in the soft tissues of the vulva and grew inwardly to involve the pelvis or originated as a primary pelvic liposarcoma. The bulk of the tumor was found to be in the pelvis at initial operation. However, the pattern of recurrence is certainly unusual for a primary pelvic sarcoma, since recurrent disease would be expected to

From the Department of Pathology and Laboratory Medicine, University of Pennsylvania and Hospital of the University of Pennsylvania.
Received for publication July 11, 1986; accepted July 28, 1986.
Reprint requests: John J. Brooks, M.D., Surgical Pathology Section/Grd. Gibson, Hospital of the University of Pennsylvania, 3400 Spruce St., Philadelphia, PA 19104.
**Supported by Eastern Cooperative Oncology Group (ECOG) Grant No. CA 15488-13.*

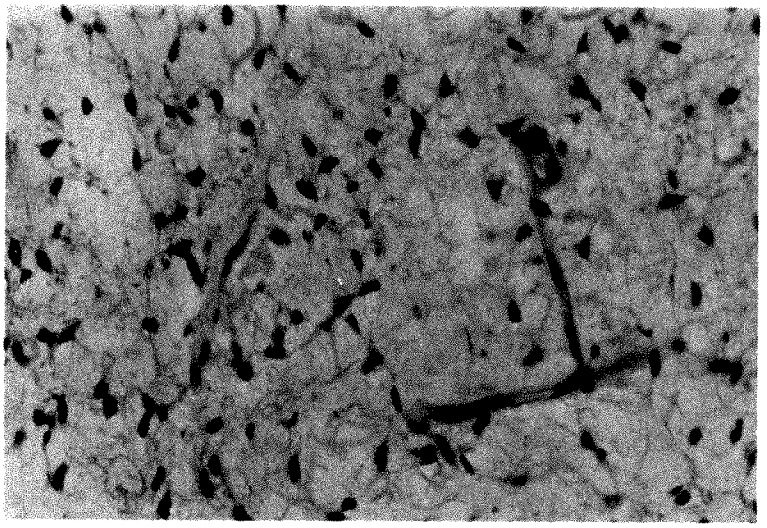


Fig. 1. Initial excision. A loose myxoid stroma with a delicate capillary network *(right)* supported uniform spindled cells with occasional vacuoles consistent with a low-grade liposarcoma.

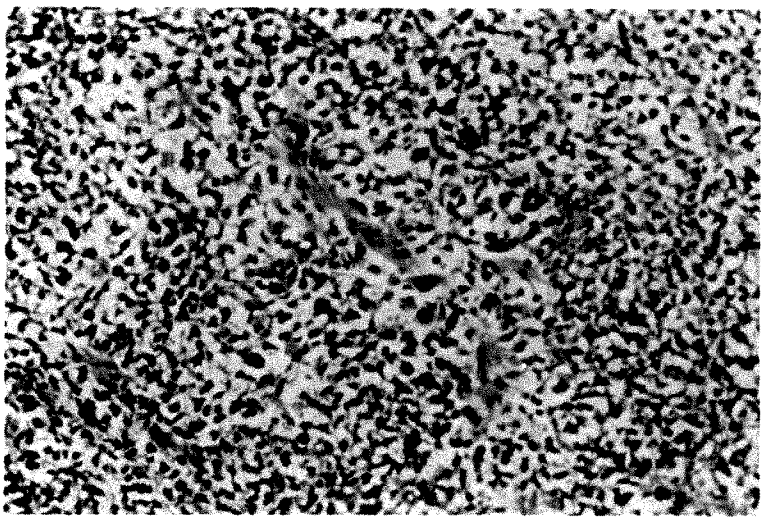


Fig. 2. Recurrence. The increased cellularity and scattered pleomorphic cells *(center)* mark this sarcoma as a high-grade round cell liposarcoma.

occur more superiorly within the retroperitoneum. In this case the tumor recurred on the left posterior medial thigh directly adjacent to the vulva. This pattern of recurrence favors the concept of a primary vulvar neoplasm.

Another unusual aspect of this case was the patient's age. Liposarcoma is a tumor of adults with a peak incidence between the fifth and seventh decades. Rare cases of childhood liposarcoma have been reported and two age peaks in the pediatric population may be seen; one at 2 years of age and the other in the early second decade (13.5 years).[2] Interestingly, there is a female predominance. The location of these pediatric tumors essentially parallels that in the adult. Seventy-six percent of the reported cases had myxoid histologic features, as in our case. In contrast to adults, children appeared to have a more favorable prognosis; the overall survival was 80% in those patients followed up for at least 18 months.[2]

Our case, apparently the first well-documented vulvar liposarcoma, was initially misdiagnosed. The low degree of cellularity, the uniform nature of the cells, and the lack of a prominent mitotic rate were misinterpreted as indicating a benign neurofibroma, a much more common vulvar tumor. However, these features as well as the prominent capillary vascular network are classic for the low-grade myxoid type of liposarcoma. One can only speculate that, had the correct diagnosis been made, radiotherapy may have prevented local recurrence and resulted in a long-term disease-free in-

terval. It is hoped that this report will call attention to the occurrence of this low-grade sarcoma on the vulva so that such confusion can be avoided in the future.

We would like to thank Dr. Arthur S. Patchefsky of Thomas Jefferson University Hospital for submitting the case in consultation with Dr. Horatio T. Enterline.

REFERENCES

1. Gompel C, Silverberg SG. Pathology in gynecology and obstetrics. Philadelphia: JB Lippincott, 1984.
2. Castleberry R, Kelly D, Wilson E, Cain W, Salter M. Childhood liposarcoma: report of a case and review of the literature. Cancer 1984;54:579-84.

Serum CA-125 antigen levels increase during menses

Donald E. Pittaway, M.D., Ph.D., and Jamil A. Fayez, M.D.
Winston-Salem, North Carolina

Serum CA-125 antigen levels were measured by radioimmunoassay before and during menses in women with and without endometriosis. Mean CA-125 levels were significantly increased in both groups during menses. Samples obtained during menses represent a potential source of elevated levels and false positive results. (Am J Obstet Gynecol 1987;156:75-6.)

Key words: CA-125 antigen, endometriosis, menstrual cycle

CA-125 is a membrane antigen that is elevated in the serum of approximately 82% of women with epithelial ovarian carcinomas[1] and is mildly elevated in some women with endometriosis.[2] While evaluating CA-125 levels in infertile women, we observed higher levels than expected when the samples were drawn during menses. Consequently, we evaluated CA-125 levels before and during menses.

Methods

Sera from 20 infertile women in whom samples were available before (cycle days 23 to 26) and during (cycle days 2 to 4) menses were analyzed retrospectively. All women had ovulatory cycles and were not receiving infertility therapy when samples were obtained. The women were divided into a nonendometriosis group and an endometriosis group (revised American Fertility Society classification, 1985) at a subsequent laparoscopy performed as part of the infertility evaluation. The mean ages (±SEM) of the two groups were 29.5 ± 0.9 for the group without endometriosis and 29.3 ± 1.1 for the group with endometriosis and were not statistically different. The etiologies of infertility in the group without endometriosis were tubal (five women), adhesions (two women), male factor (one), and unexplained (one). CA-125 was measured in the serum with a solid phase radioimmunoassay according to the manufacturer's instructions (Abbott Laboratories, North Chicago, Illinois). A determination of the serum β-sub-

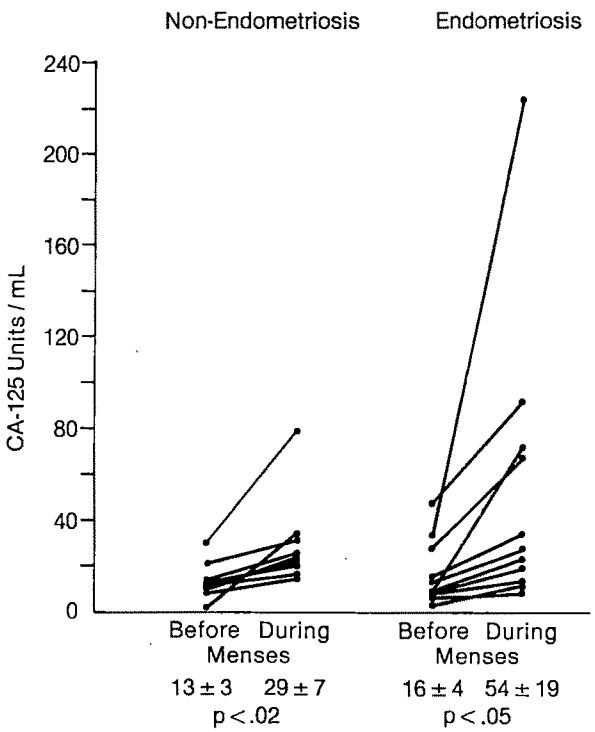


Fig. 1. Serum CA-125 levels before and during menses in women without endometriosis (n = 9) and with endometriosis (n = 11). The mean ± SEM values are indicated below each group.

From the Section on Reproductive Endocrinology, Department of Obstetrics and Gynecology, Bowman Gray School of Medicine.
Received for publication February 21, 1986; revised June 30, 1986; accepted July 7, 1986.
Requests: Donald E. Pittaway, M.D., Ph.D., Department of Obstetrics and Gynecology, Bowman Gray School of Medicine, 300 South Hawthorne Road, Winston-Salem, NC 27103.

unit of human chorionic gonadotropin (Clinetics Corporation, Tustin, California) was performed on each sample to exclude an occult pregnancy. The data were evaluated by paired and unpaired *t* tests.

Results

As shown in Fig. 1, the mean CA-125 levels were significantly higher during menses in both the group without endometriosis ($p < 0.02$) and the group with endometriosis ($p < 0.05$). In both groups, all CA-125 levels before menses were less than 65 U/ml. In the group without endometriosis one of nine women (11%) and in the group with endometriosis four of 11 women (36%) had CA-125 levels >65 U/ml during the menses.

Comment

In 988 nonpregnant patients attending a gynecologic clinic, 11 (1.1%) had elevated CA-125 levels (>65 U/ml).[3] Since a large proportion of these patients (n = 652) were presenting for an annual checkup and since women nearly always arrange a gynecologic visit while not menstruating unless vaginal bleeding has been a problem, most of the women in that series would not be expected to be menstruating.

In the present study of a selected group of infertile women, a higher CA-125 level was observed during menses, and five of 20 women (25%) had elevated levels (>65 U/ml) during but not before menses. An increase in CA-125 levels during menses has also been observed in rhesus monkeys with and without endometriosis.[4] We conclude that CA-125 determinations performed during the menses, particularly in women with endometriosis, represent a source of higher values and false positive results.

REFERENCES

1. Bast RC Jr, Klug TL, St John E, et al. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N Engl J Med 1983;309:883.
2. Barbieri RL, Bast RC, Niloff JM, Kistner RW, Knapp RC. Elevated serum concentrations of CA-125 in patients with advanced endometriosis. Fertil Steril 1986;45:630.
3. Niloff JM, Knapp RC, Schaetzl E, Reynolds C, Bast RC Jr. CA-125 antigen levels in obstetrics and gynecologic patients. Obstet Gynecol 1984;64:703.
4. Pittaway DE, Klimek M, Koritnik DR. CA-126 antigen in rhesus monkeys with spontaneous endometriosis. Fertil Steril (in press).

Advanced ultrasonic placental maturation in twin pregnancies

G. Ohel, M.D., M. Granat, M.D., D. Zeevi, M.D., A. Golan, M.D., Sh. Wexler, M.D., M. P. David, M.D., and J. G. Schenker, M.D.

Jerusalem and Tel Aviv, Israel

The mean length of twin pregnancies is shorter than that of singleton pregnancies. The possibility that the shorter gestation of twins is associated with advanced fetal maturational changes was studied in relation to placental maturation. For this purpose, the sonographically determined placental gradings of 158 twin pregnancies and 474 singleton pregnancies were compared at different gestational ages. The percent distributions of placental grades, from I to III, were significantly different throughout the third trimester, with a preponderance of Grade III placentas in the twin group ($p < 0.001$). Considering the reported association of Grade III placentas with advanced gestation as well as fetal lung maturity, the present study suggests earlier maturational changes in twin fetuses compared with singleton fetuses. (Am J Obstet Gynecol 1987;156:76-8.)

Key words: Sonographic placental maturation, twin pregnancies

Advances in ultrasonic imaging have enabled the characterization of placental restructuring throughout gestation. A grading system, from 0 to 3, has been proposed to describe placental maturational changes,[1] and these have been correlated to fetal pulmonary maturity.[1-4]

Since normal twin gestations are commonly shorter than singleton pregnancies,[5] it may be anticipated that maturational processes, including those involved in fetal lung development, occur at an earlier gestational age in twins than in singleton fetuses. The present study has attempted to investigate this possibility by compar-

From the Department of Obstetrics and Gynecology, Hadassah University Hospital, Jerusalem, and the Department of Obstetrics and Gynecology "B," Serlin (Hakirya) Maternity Hospital, Tel Aviv.
Received for publication April 1, 1986; accepted July 30, 1986.
Reprint requests: G. Ohel, M.D., Department of Obstetrics and Gynecology, Hadassah University Hospital, P. O. B. 12000, Jerusalem 91120, Israel.

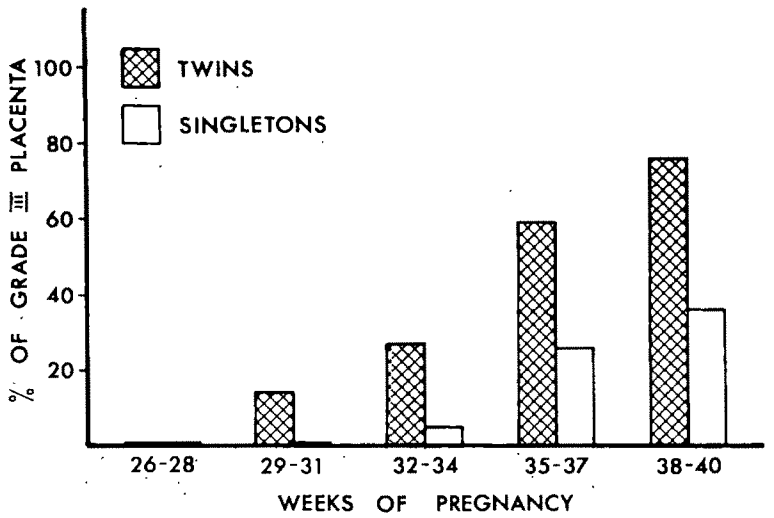


Fig. 1. The percentage of Grade III placentas in twin and singleton pregnancies, compared at different gestational ages ($p < 0.001$ for all age groups).

Table I. The percent distribution of placental grades in twin and singleton pregnancies

	Twins				*Singletons*				
Weeks' gestation	*No. of patients*	*Placental Grade (%)*			*No. of patients*	*Placental Grade (%)*			*p Value*
		I	*II*	*III*		*I*	*II*	*III*	
38-40	21	0	23.8	76.2	63	0	63.5	36.5	<0.01
35-37	44	9.1	31.8	59.1	132	15.2	58.3	26.5	<0.001
32-34	37	32.4	40.6	27	111	42.3	53.2	4.5	<0.001
29-31	35	42.9	42.9	14.2	105	66.7	33.3	0	<0.001
26-28	13	61.5	38.5	0	39	74.4	25.6	0	NS
23-25	8	87.5	12.5	0	24	100	0	0	NS

ing the sonographic placental grading at different gestational ages in both twin and singleton pregnancies.

Material and methods

A retrospective analysis was carried out on records of routine real-time ultrasonic examinations of twin and singleton pregnancies. In all studied cases, the gestational ages were known through the "last menstrual period" or early ultrasound examinations. Cases were omitted when discrepancies of more than 2 weeks were noted between the clinical and sonographically determined gestational ages. Also not considered were cases with medical or obstetric complications. A total of 158 twins were studied, each with one ultrasonic examination, at gestational ages that ranged from 23 weeks to term. For each twin pregnancy, three consecutively performed ultrasound examinations of singleton pregnancies, matched for gestational age, served as controls.

Ultrasound examinations were performed with the use of linear array real-time scanners with 3.5 MHz transducers. Placental maturation was evaluated with the use of the grading classification of Grannum et al.[1] Where an equivocal grading was given, the more advanced grade was assigned to that case.

The χ^2 test was used to analyze the difference in the distribution of placental Grades I to III at the different gestational ages in the two study groups. A logistic regression analysis, with the Bio Medical Program Logistic Regression (BMDPLR) computer program, was used to demonstrate the difference between singleton and twin pregnancies with regard to the proportion of cases with Grade III placentas at the different gestational ages.

Results

The percent distribution of placental gradings, from I to III, in the twin and singleton pregnancies is shown in Table I. From 29 weeks to term the percent distribution of placental gradings showed a statistically significant difference when the two groups were compared, with a preponderance of Grade III placentas in the twin group. No differences were apparent at the earlier gestational ages of 23 to 28 weeks.

The proportion of Grade III placentas in the twin and singleton groups is shown in Fig. 1.

While the proportion of Grade III placentas was increased with advancing gestation in both groups, it was significantly greater in the twins at each gestational age group from 29 weeks to term ($p < 0.001$).

Comment

The key finding of this study is the demonstration of an advanced sonographic maturation of twin placentas as compared with singleton placentas. Grade III placentas were first noted in twins of 29 to 31 weeks' gestation, while this grade was absent in singleton pregnancies of comparable gestational ages. Thereafter, at each 3-week interval to term, Grade III placentas were significantly more common in twins compared with singletons ($p < 0.001$).

Previous studies have demonstrated that the placenta undergoes a series of progressive morphologic changes, observed by ultrasonography, and these are related to gestational age and fetal lung maturation.[1-4] That gestational age is not the only determinant of placental grading is well illustrated by our finding of a more advanced placental maturation in twins compared with singletons of similar gestational ages. This may point to a difference in the rate of maturation dynamics of twins and singletons. In this respect our findings are in accord with, and complementary to, a recent study of the amniotic fluid lecithin/sphingomyelin ratio, which demonstrated fetal lung maturation to occur several weeks earlier in twins compared with singletons.[6] A possible explanation of the observed differences in placental maturation may be related to the different intrauterine growth pattern of singleton and twin fetuses.[7] The altered growth potential of twins may parallel the situation of growth retardation in singleton fetuses. It has been suggested that intrauterine growth is an important determinant of sonographically diagnosed placental maturity and that for the small fetus a mature (Grade III) placeta is significantly associated with growth retardation.[8]

In the case of singleton pregnancies, it is very unlikely for a neonate to develop hyaline membrane disease in the presence of a Grade III placenta near term.[1-4] Nevertheless, this view is not shared by all, and one report has concluded that placental grading is not accurate enough to replace amniocentesis as the standard test of fetal pulmonary maturity.[9] As the presence of a Grade III placenta is affected by both gestational age and pregnancy complications,[8, 10] at least in a subgroup of preterm complicated pregnancies, sonographic grading of the placenta as Grade III may still be associated with hyaline membrane disease.[10] With this in mind and until data that correlate placental grading of twins with hyaline membrane disease become available, it would seem unjustified in twin pregnancies to rely solely on placental grading as an indicator of fetal pulmonary maturity.

We thank Mr. Israel Einot of the statistical consultation service at the Hebrew University, Mount Scopus, Jerusalem, Israel.

REFERENCES

1. Grannum PAT, Berkowitz RL, Hobbins JC. The ultrasonic changes in the maturing placenta and their relation to fetal pulmonic maturity. AM J OBSTET GYNECOL 1979; 133:915.
2. Hobbins JC. Use of ultrasound in complicated pregnancies. Clin Perinatol 1980;7:397.
3. Petrucha RA, Golde SH, Platt LD. Real-time ultrasound of the placenta in assessment of fetal pulmonic maturity. AM J OBSTET GYNECOL 1982;142:463.
4. Kazzi GM, Gross TL, Sokol RJ, Kazzi SNJ. Noninvasive prediction of hyaline membrane disease: an optimized classification of sonographic placental maturation. AM J OBSTET GYNECOL 1985;152:213.
5. Ho SK, Wu PYK. Perinatal factors and neonatal morbidity in twin pregnancy. AM J OBSTET GYNECOL 1975;122:979.
6. Leveno KJ, Quirk JG, Whalley PJ, Herbert WNP, Trubey R. Fetal lung maturation in twin gestation. AM J OBSTET GYNECOL 1984;148:405.
7. Gruenwald P. Environmental influences on twins apparent at birth. A preliminary study. Biol Neonate 1970; 15:79.
8. Kazzi GM, Gross TL, Sokol RJ, Kazzi NJ. Detection of intrauterine growth retardation: a new use for sonographic placental grading. AM J OBSTET GYNECOL 1983; 145:733.
9. Harman CR, Manning FA, Stearns E, Morrison I. The correlation of ultrasonic placental grading and fetal pulmonary maturation in five hundred sixty-three pregnancies. AM J OBSTET GYNECOL 1982;143:941.
10. Kazzi GM, Gross TL, Rosen MG, Kazzi NYJ. The relationship of placental grade, fetal lung maturity, and neonatal outcome in normal and complicated pregnancies. AM J OBSTET GYNECOL 1984;148:54.

Antenatal sonography of fetal malformations associated with drugs and chemicals: A guide

Gideon Koren, M.D., A.B.M.T., Michelle Brille Edwards, M.D., and Murray Miskin, M.D.
Toronto, Ontario, Canada

A guide presented in this article provides the sonographer with a list of fetal malformations that have been described in association with specific drugs or chemicals and that can be visualized by current ultrasonographic techniques. (AM J OBSTET GYNECOL 1987;156:79-85.)

Key words: Ultrasonography, fetal malformations, teratogens

Since the thalidomide disaster in the 1950s, an increasing number of drugs and chemicals have been incriminated as causing teratogenicity or fetal toxicity. As the majority of pregnant women are exposed to at least one medication during pregnancy either before or after realizing they are pregnant,[1] the need to determine the relative risk that a certain agent will cause malformations is of utmost importance.

Data on chemically induced teratogenic risk are derived from animal studies, human case reports, or epidemiologic studies. While such information is essential for assigning a relative risk to a potential teratogen, it may be of little help for medication in a specific case. Sonography has emerged as a powerful tool for antenatal detection of fetal anomalies. It is conceivable therefore that more and more pregnant women exposed to drugs and chemicals will be referred for diagnosis of or to rule out malformations associated with these agents. Currently, pregnant women who attend our Motherisk Clinic for antenatal counseling of drug-chemical exposure are scheduled for a level 2 sonographic evaluation at 17 to 18 weeks of gestation.

The guide aims at providing the sonographer with a practical list of malformations that have been described in association with specific drugs or chemicals.

Methodologic considerations

Two groups of agents are included. (1) Common drugs and chemicals that have been associated with malformations are listed. In many cases the incriminating data are controversial; hence the inclusion of a certain malformation in this guide by no means suggests that we are convinced that the agent is a teratogen. Because of the heterogeneity of the data, the source of information is mentioned (for instance, case reports, retrospective or prospective studies). (2) Drugs and chemicals that have been proved beyond doubt as teratogens are listed.

Table I (see pages 80-85) includes only malformations that can be visualized by current ultrasonographic techniques and cannot be used as a complete list of drug-induced teratogenicity. The data in this guide have been extracted from currently available literature.[1-6]

Certain points with respect to the ultrasound examination itself should be stressed. It is probably best carried out by a physician ultrasonographer who has had significant experience in looking at fetuses. A very meticulous technique and high-resolution real-time equipment should be used. The examination is best recorded on videotape so that it can be reviewed as needed. A great deal of patience is required in this type of study, especially when one is examining the face, digits, and heart. At the outset of the examination it should be explained to the patient that the examination may take some time to complete and that more than one sitting may be required.

From the Motherisk Clinic, the Division of Clinical Pharmacology, the Research Institute, and the Department of Pediatrics, The Hospital for Sick Children, and the Department of Pharmacology and Pediatrics, University of Toronto.
Received for publication June 3, 1986; accepted July 22, 1986.
Reprint requests: Dr. Gideon Koren, Division of Clinical Pharmacology, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8.

REFERENCES

1. Schandein J. Chemically induced birth defects. New York: Marcel Dekker, 1985.
2. Fabro S, ed. Reproductive toxicology. A medical letter. Washington DC: Reproductive Toxicology Center.
3. Mattison DR, ed. Reproductive toxicology. New York: Alan R Liss, 1983.
4. Briggs GG, Bodendorier TW, Freeman RK, Yaffe SJ. Drugs in pregnancy and lactation. 2d ed. Baltimore: Williams & Wilkins, 1986.
5. Heinonen OP, Slone D, Shapiro S. Birth defects and drugs in pregnancy. Littleton, Massachusetts: PSG Publishing Co, 1977.
6. Shepard TH. Catalog of teratogenic agents. 4th ed., Baltimore: Johns Hopkins University Press, 1983.

Table I. Common drugs and chemicals described in association with fetal malformations, only some of which are established teratogens

Drug	*Central nervous system*	*Cardiovascular*	*Skeleton*
Acetaminophen (overdose)			
Acetazolamide			Sacrococcygeal teratoma
Acetylsalicyclic acid		Intracranial hemorrhage	
Albuteral		Fetal tachycardia	
Alcohol	Microcephaly		Short nose, hypoplastic maxilla, micrognathia, occasional features of skeleton
Amantadine		Single ventricle with pulmonary atresia	
Aminopterin*	Meningoencephalocele, hydrocephalus, incomplete skull ossification, brachycephaly, anencephaly		Hypoplasia of thumb and fibula, clubfoot, syndactyly, hypognathia
Amitriptyline			Micrognathia
Amobarbital	Anencephaly	Congenital heart malformations	
Antithyroid drugs*			
Azathioprine*		Pulmonary valvular stenosis	
Betamethasone	Reduced head circumference		
Bromides			
Busulfan			
Caffeine			Musculoskeletal defects
Captopril			
Carbon monoxide*	Cerebral atrophy, hydrocephalus		
Carbamazepine	Meningomyelocele	Atrial septal defect, patent ductus arteriosus	Nose hypoplasia, hypertelorism
Chlordiazepoxide	Microcephaly	Congenital defects of heart	
Chloroquine			
Chlorpheniramine	Hydrocephalus		
Chlorpropamide	Microcephaly		
Clomiphene	Meningomyelocele, hydrocephalus, microcephaly, anencephaly		
Codeine	Hydrocephalus	Congenital cardiac defects	Musculoskeletal malformations
Cortisone	Hydrocephalus	Ventricular septal defect, coarctation of aorta	
Coumadin*	Encephalocele, anencephaly, spina bifida	Congenital heart disease	Nasal hypoplasia, scoliosis, skeletal deformities
Cyclophosphamide*	Tetralogy of Fallot		Flattened nasal bridge
Cytarabine*	Anencephaly	Tetralogy of Fallot	
Daunorubicin*	Anencephaly	Tetralogy of Fallot	
Dextroamphetamine	Exencephaly	More cardiac defects than controls, atrial septal defect	

Since only malformations that can be visualized by current ultrasonographic techniques are listed, the guide cannot be used as a complete list of drug-induced teratogenicity. CR = Case reports; RS = retrospective studies; PS = prospective studies; AS = animal studies.

*Proved to be teratogenic.

Extremities	*Gastrointestinal*	*Genitourinary*	*Miscellaneous*	*Source*
			Polyhydramnios	CR
				CR
			Growth retardation	CR
				RS
				PS
				PS
			Growth retardation	CR
				RS
				PS
				CR
				CR
Limb reduction, swelling of hands and feet		Urinary retention		CR
Severe limb deformities, congenital hip dislocation, polydactyly, clubfoot	Oral cleft	Intersex	Soft tissue deformity of neck	CR PS RS
			Goiter	CR RS
Polydactyly				CR AS
Polydactyly, clubfoot, congenital dislocation of hip				CR PS
	Pyloric stenosis, cleft palate		Microphthalmia, growth retardation	CR
		Hydronephrosis		PS
Leg reduction				CR
			Stillbirth	CR
Congenital hip dislocation	Cleft lip			CR PS RS
	Duodenal atresia			RS
			Hemihypertrophy	CR
Polydactyly, congenital dislocation of hip				PS
Dysmorphic hands and fingers				CR
Syndactyly, clubfoot, polydactyly	Esophageal atresia			CR RS
Dislocated hip	Pyloric stenosis, oral cleft		Respiratory malformations	PS RS
Clubfoot	Cleft lip			CR
Stippled epiphysis, chondroplasia punctata, short phalanges, toe defects	Incomplete rotation of gut		Growth retardation, bleeding	CR RS
Four toes on each foot, hypoplastic midphalanx, syndactyly				CR
Lobster claw of 3 digits, missing feet digits, syndactyly				CR
Syndactyly			Growth retardation	CR CR RS

continued

Table I—Cont'd

Drug	*Central nervous system*	*Cardiovascular*	*Skeleton*
Diazepam	Spina bifida	More cardiac defects than controls	
Diphenhydramine			
Disulfiram			Vertebral fusion
Diuretics			
Estrogens		Congenital cardiac malformation	
Ethanol*	Microcephaly	Ventral septal defect, atrial septal defect, double outlet of right ventricle, pulmonary atresia, dextrocardia, patent ductus arteriosus, tetralogy of Fallot	Short nose, hypoplastic philtrum, micrognathia, pectus excavatum, radioulnar synostosis, bifid xyphoid, scoliosis
Ethosuximide	Hydrocephalus		Short neck
Fluorouracil			
Fluphenazine			Poor ossification of frontal bone
Haloperidol			
Heparin*			
Hormones, progestogenic	Anencephaly, hydrocephalus	Tetralogy of Fallot, truncus arteriosus, ventral septal defect	Spina bifida
Imipramine	Exencephaly		
Indomethacin			
Isoniazid	Meningomyelocele		
Lithium	Hydrocephalus, meningomyelocele	Ventral septal defect, Ebstein's anomaly, mitral atresia, patent ductus arteriosus, dextrocardia	Spina bifida
Lysergic acid diethylamide	Hydrocephalus, encephalocele, meningomyelocele		
Meclizine		Hypoplastic left heart	
Meprobamate		Congenital heart malformations	
Methotrexate*	Oxycephaly, absence of frontal bone, large fontanelles	Dextrocardia	Hypoplastic mandible
Methyl mercury*	Microcephaly, asymmetric head		
Metronidazole			Midline facial defects
Nortriptyline			
Oral contraceptives	Meningomyelocele, hydrocephalus, anencephaly	Cardiac anomalies	Vertebral malformations
Parameth-adione		Tetralogy of Fallot	
Penicillamine		Ventral septal defect	
Phenobarbital	Hydrocephalus, meningomyelocele		
Phenothiazines	Microcephaly		
Phenylephrine			Eye and ear abnormalities
Phenylpropanolamine			Pectus excavatus
Phenytoin*	Microcephaly, wide fontanelles	Congenital heart malformation	Rib-sternal abnormalities, short nose, broad nasal bridge, wide fontanelle, broad alveolar ridge, short neck, hypertelorism, low-set ears

Extremities	*Gastrointestinal*	*Genitourinary*	*Miscellaneous*	*Source*
Absence of arm, syndactyly, absence of thumbs	Cleft lip-palate			CR RS
Clubfoot	Cleft palate			PS CR
Clubfoot, radial aplasia, phocomelia	Tracheoesophageal fistula			CR
			Respiratory malformations	PS
Limb reduction				CR PS
	Oral cleft		Growth retardation, diaphragmatic hernia	RS PS
	Oral cleft			CR
Radial aplasia, absent thumbs	Aplasia of esophagus and duodenum		Hypoplasia of lungs	CR
	Oral cleft			CR
Limb deformities				RS
			Bleeding	CR RS
Absence of thumbs				CR
Limb reduction	Cleft palate	Renal cystic degeneration	Diaphragmatic hernia	CR
Phocomelia			Stillbirth, hemorrhage	CR
				CR CR PS
Limb deficiencies				CR RS
			Respiratory defects	RS
Bilateral defects of limbs				RS CR
Long webbed fingers			Growth retardation, low-set ears	CR
				RS CR CR
Limb reduction				CR
Limb reduction	Tracheoesophageal malformations		Growth retardation	CR RS
			Growth retardation	CR
	Pyloric stenosis		Growth retardation	CR
Digital anomalies	Cleft palate, ileal atresia		Growth retardation, pulmonary hypoplasia	CR
Syndactyly, clubfoot	Omphalocele, abdominal distention			CR PS
Syndactyly, clubfoot, congenital dislocation of hip	Umbilical hernia			PS
Polydactyly, congenital dislocation of hip				PS
Hypoplastic distal phalanges, digital thumb, dislocated hip	Cleft palate-lip		Growth retardation	CR RS

continued

Table I—Cont'd

Drug	*Central nervous system*	*Cardiovascular*	*Skeleton*
Polychlorinated biphenyls*			Spotted calcification in skull, fontanelle and sagittal suture
Primidone		Ventral septal defect	Webbed neck, small mandible
Procarbazine*	Cerebral hemorrhage		
Quinine	Hydrocephalus	Congenital heart defects	Facial defects, vertebral anomalies
Retinoic acid*	Hydrocephalus, microcephaly	Various congenital heart defects	Malformations of cranium, ear, face, ribs
Spermicides			
Sulfonamide			
Tetracycline			
Thalidomide*		Congenital heart malformations	Spine malformation
Thioguanine			
Tobacco			
Tolbutamide			
Trifluoperazine		Transposition of great arteries	
Trimethadione*	Microcephaly	Atrial septal defect, ventral septal defect	Low-set ears, broad nasal bridge
Valproic acid*	Lumbosacral meningomyelocele, microcephaly, wide fontanelle	Tetralogy of Fallot	Depressed nasal bridge, hypoplastic nose, low-set ears, small mandibles

Extremities	*Gastrointestinal*	*Genitourinary*	*Miscellaneous*	*Source*
			Stillbirth, growth retardation	PS
				RS
				CR
Oligodactyly				CR
Dysmelias				CR
Limb deformities			Stillbirth	RS
				PS
Limb reduction				RS
Hypoplasia of limb or part of it, foot defects		Urethral obstructions		PS
Hypoplasia of limb or part of it, clubfoot				CR
				PS
Limb reduction (amelia, phocomelia), hypoplasia	Duodenal stenosis or atresia, pyloric stenosis		Microtia	RS
				PS
Missing digits				CR
			Growth retardation	PS
				RS
Finger-toe syndactyly, absent toes, accessory thumb				CR
Phocomelia				CR
Malformed hands, clubfoot	Esophageal atresia		Growth deficiency	CR
				PS
	Oral cleft		Growth deficiency	CR

Fryns syndrome: A predictable, lethal pattern of multiple congenital anomalies

Arnon Samueloff, M.D., Daniel Navot, M.D., Arie Birkenfeld, M.D., and Joseph G. Schenker, M.D.
Jerusalem, Israel

Fryns syndrome is a unique pattern of lethal multiple congenital malformations with variable expression. A family in which all four sibs conformed to Fryns syndrome is detailed and substantiates the criteria for definition of the syndrome; perinatal mortality, hypoplastic lungs, and facial deformities should be highly suggestive of the syndrome. The addition of a strong family history, diaphragmatic hernias, distal limb deformities, and early onset of polyhydramnios with subsequent premature delivery should definitely confirm the diagnosis. (Am J Obstet Gynecol 1987;156:86-8.)

Key words: Fryns syndrome, multiple congenital anomalies, hypoplastic lungs, distal limb malformation, facial deformities

Recently a new lethal autosomal recessive syndrome of multiple congenital anomalies, with familial occurrence, has been defined.[1-3] In 1979, Fryns et al.[1] reported an instance of two female sibs with a unique malformation pattern including multiple facial anomalies, diaphragmatic defects, hypoplastic lungs, and distal limb deformities. Later the same group added an additional case with a similar pattern.[2] Lubinsky et al.[3] contributed two additional cases and termed the malformation pattern Fryns syndrome. Recently we encountered a family in which all four sibs conformed to Fryns syndrome, the last of which was diagnosed in utero.

The four additional cases of Fryns syndrome are reported in order to further delineate the variability within the syndrome. The extended series establishes a malformation pattern that, together with the strong family history, should alert the perinatologist for a possible recurrence.

Family history

The parents were healthy, unrelated Sepharadic Jews, aged 31 and 28 years. The mother had had five pregnancies; in one, spontaneous abortion occurred during the first trimester, while four pregnancies advanced into the third trimester of gestation. All four were complicated by the early onset of polyhydramnios and terminated in premature deliveries of three boys and a girl who had the major characteristicis of Fryns syndrome and died shortly after birth. Table I details pregnancy and delivery characteristics of the four siblings, as well as major anomalies according to the systems affected. The four most consistent findings are polyhydramnios, facial deformities, diaphragmatic hernia, and hypoplastic lungs.

Prior to the last pregnancy, a search for possible etiologic factors of the recurrent malformation pattern was performed: glucose tolerance test, Sabin-Feldman test, karyotyping of both parents, VDRL test, and anticytomegalovirus antibodies gave no clue as to a possible cause. The last pregnancy was uneventful until the twenty-sixth week, when rapidly advancing polyhydramnios was detected. The volume of amniotic fluid stabilized toward the twenty-ninth week, after repeated amniocenteses. Ultrasonography revealed sonolucent areas adjacent to the fetal heart, a picture consistent with dilated loops of bowel inside the thoracic cavity. In the thirty-first week of gestation, after premature rupture of the membranes and in view of a previous cesarean section, a repeat cesarean section was performed. A 2000 gm female infant with multiple congenital anomalies was delivered (Fig. 1, *A* and *B*). The infant died within a few minutes after delivery. Autopsy revealed multiple facial defects, agenesis of the diaphragm, bilaterally atrophic kidneys, distal limb deformities, and some other anomalies detailed in Table I.

Comment

Up to now, five cases of Fryns syndrome have been reported in the literature; the present series adds four additional cases. The marked variability within the syndrome necessitates the description of each and every affected fetus until final criteria are set up for definition

From the Department of Obstetrics and Gynecology, Hadassah University Hospital.
Received for publication February 17, 1986; revised June 30, 1986; accepted July 12, 1986.
Reprint requests: A. Samueloff, M.D., Department of Obstetrics and Gynecology, Hadassah University Hospital, P. O. B. 12000, Jerusalem 91120, Israel.

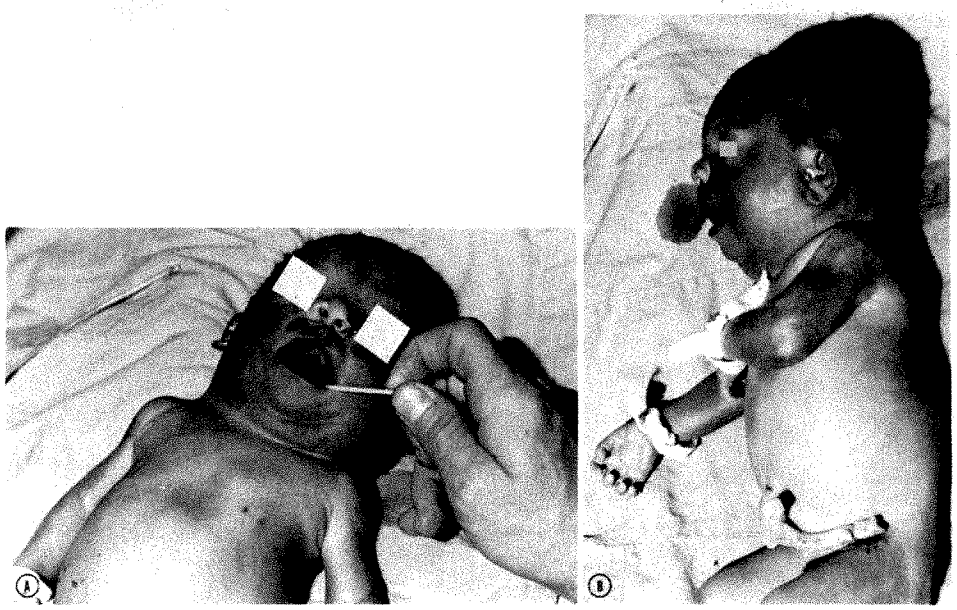


Fig. 1. The propositus with Fryns syndrome. Cleft palate, micrognathia, retrognathia, coarse facies, and small thorax are evident.

Table I. Details of pregnancy, fetal anomalies, and outcome in five reported cases of Fryns syndrome and the additional four cases of the present series

	Fryns et al.,[1] *1979*		*Goddeeris et al.,*[2] *1980*	*Lubinsky et al.,*[3] *1983*		*Present series*			
	No. 1	*No. 2*	*(No. 3)*	*No. 4*	*No. 5*	*No. 6*	*No. 7*	*No. 8*	*No. 9*
Polyhydramnios	+	+	+	–	–	+	+	+	+
Gestational age at onset of polyhydramnios	24	29	<28	–	–	25	25	24	25
Gestational age at delivery (wk)	28	29	32	41	39	33	34	34	31
Fetal weight at delivery (gm)	1200	1600	1360	4780	4030	2200	?	3000	2000
Family history	+	+	–	–	+	+	+	+	+
Fetal anomalies									
Perinatal mortality	+	+	+	+	+	+	+	+	+
Lung hypoplasia	+	+	+	+	+	+	+	+	+
Diaphragmatic defect	+	+	+	–	+	+	+	+	+
Coarse facies	+	+	+	–	–	+	+		+
Broad nasal bridge	+	–	+	+	+	+	?	?	+
Cleft palate	+	–	+	+	+	+	?	+	+
Micrognathia, retrognathia	+	+	+	+	+	?	?	?	+
Cloudy cornea	+	+	–	–	–	?	?	?	+
Small thorax	+	+	–	–	–	?	?	?	+
Hypoplastic nipples	+	+	+	–	–	?	?	?	+
Distal limb hypoplasia	+	+	+	+	+	+	?	?	+
Genital anomalies	+	–	+	+	+	?	?	+	+
Kidney anomalies	–	–	–	+	+	?	+	?	+
Karyotype of fetus	46,XX		46,XX				46,XY		46,XX
Sex of fetus	Female	Female	Female	Female	Male	Male	Male	Male	Female

of the syndrome. We have compiled in Table I all the malformations encountered in the nine reported cases, as well as pregnancy and delivery characteristics. Cases Nos. 3, 6, and 9 conform to the original pattern described by Fryns et al.[1] (Cases 1 and 2). Our two additional cases (Nos. 7 and 8) are concordant in the early onset of polyhydramnios, premature delivery, and the presence of diaphragmatic hernias and hypoplastic lungs. Some of the missing features, such as distal limb deformities, may be attributed to the scarcity of information available for those two cases. The pattern of multiple congenital anomalies in the extended series still conforms to the syndrome originally described by Fryns et al.[1] Table II details the concordant features in

Table II. Concordant features in nine cases of Fryns syndrome

Feature	*No. of cases*
Perinatal mortality	9
Lung hypoplasia	9
Facial deformations	9
Family history	8
Diaphragmatic defects	8
Distal limb hypoplasia	7
Early onset of polyhydramnios	7
Premature delivery	7
Genital anomalies	6
Hypoplastic nipples	4
Cloudy cornea	3
Small thorax	3

the nine cases. The uniformly reported perinatal mortality, hypoplastic lungs, and facial deformities should be highly suggestive of Fryns syndrome. A strong family history, diaphragmatic hernias, distal limb deformities, and early onset of polyhydramnios with subsequent premature delivery should probably be added as minimal criteria for the definition of the syndrome (Table II).

The information now available should suffice for the prediction of the syndrome in future sibs. A thorough prenatal evaluation by ultrasonography, with or without amniography, may detect the polyhydramnios, diaphragmatic hernia, hypoplastic lungs, renal anomalies, and distal limb anomalies. A positive prenatal diagnosis may influence clinical decision making as to the route and timing of delivery.

REFERENCES

1. Fryns JP, Moerman F, Goddeeris P, Bossuyt C, Van den Berghe H. A new syndrome with cloudy cerneae diaphragmatic defects and distal limb deformities. Hum Genet 1979;50:65.
2. Goddeeris P, Fryns JP, Van den Berghe H. Diaphragmatic defects, craniofacial dysmorphism, cleft palate and distal limb deformities—a new lethal syndrome. J Genet Hum 1980;28:57.
3. Lubinsky M, Severn C, Rapoport JM. Fryns syndrome: A new variable multiple congenital anomaly (MCA) syndrome. Am J Med Genet 1983;14:461.

Unruptured pregnancy in a heterotopic fallopian tube: Evidence for transperitoneal sperm migration

Charles Brown, M.D., William E. LaVigne, M.D., and Santiago L. Padilla, M.D.
Augusta, Georgia

A case of an unruptured pregnancy in a heterotopic left fallopian tube isolated from a unicornuate uterus with a normal contralateral tube and ovary is presented. The corpus luteum of pregnancy was in the left ovary. This case provides supportive evidence for transperitoneal sperm migration. (Am J Obstet Gynecol 1987;156:88-90.)

Key words: Ectopic pregnancy, müllerian anomaly, sperm migration

Congenital anomalies of the müllerian ducts have been estimated to occur in 1 in 500 to 1 in 2000 women.[1] The most common are the uterine anomalies. Structural anomalies of the fallopian tubes are very rare. Anomalies reported include complete absence of the tube and ovary, unilateral müllerian duct development (uterus unicornis), maldevelopment together with failure of fusion of the ducts, duplication of the tube, and absence of tubal segments. Ectopic pregnancy in heterotopic fallopian tubes is extremely rare.[1, 2]

From the Department of Obstetrics and Gynecology, Medical College of Georgia, and the Department of Obstetrics and Gynecology, University Hospital.
Received for publication March 24, 1986; revised June 16, 1986; accepted July 7, 1986.
Reprint requests: Charles Brown, M.D., Department of Obstetrics and Gynecology, Medical College of Georgia, Augusta, GA 30912.

A case of an unruptured pregnancy in a heterotopic fallopian tube associated with a unicornuate uterus is presented. It provides evidence for the phenomenon of transperitoneal sperm migration.

Case presentation

A 24-year-old primigravid woman presented at 7 weeks' menstrual age with mild vaginal bleeding. She had had a positive human chorionic gonadotropin radioreceptor assay approximately 2 weeks earlier. On examination, the abdomen was soft and there was a small amount of bright red blood in the vaginal vault. Bimanual examination revealed a slightly enlarged uterus and no palpable adnexal masses. Three days later she had continued bleeding without abdominal pain and complained of having passed some tissue from the vagina. Physical examination was essentially un-

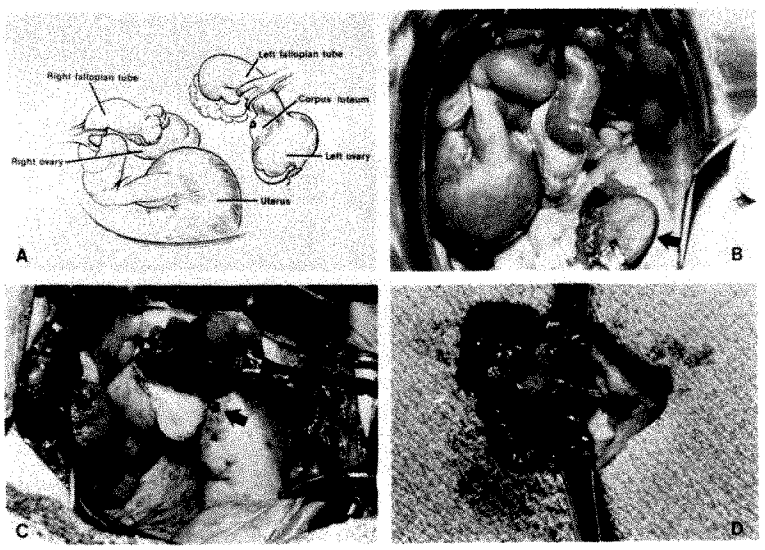


Fig. 1. *A*, Diagram of intraoperative findings. *B*, The unicornuate uterus with a normal right fallopian tube and ovary. Part of the heterotopic left fallopian tube is seen *(arrow)*. *C*, Heterotopic fallopian tube with a corpus luteum *(arrow)*. *D*, A small gestational sac in the ampulla of the heterotopic tube.

changed except for increased tenderness of the uterus and adnexa. An ultrasound examination revealed an empty uterus, a left-sided complex mass posterior to the uterus, and fluid in the cul-de-sac. A serum titer of the β-subunit of human chorionic gonadotropin was 147 mIU/ml. A culdocentesis produced 22 ml of bright red nonclotting blood.

The patient's past medical history was significant for having had a left inguinal hernia repaired 6 months before admission.

At laparotomy, a slightly enlarged hemiuterus was noted (Fig. 1). The right fallopian tube, round ligament, broad ligament, and ovary appeared to be normal. A heterotopic left fallopian tube and ovary were found attached to the pelvic sidewall. This fallopian tube was 4 to 5 cm in length and consisted of an ampulla and a well-developed fimbriated end. The ampulla was distended and active bleeding from the fimbriated end was noted. Careful exploration failed to disclose any connection between the heterotopic fallopian tube and the uterus. The left fallopian tube was resected and the left ovary, which contained the corpus luteum, was left intact. The tube was opened and it contained a gestational sac. Microscopic examination of the tube revealed a hematosalpinx and ghost villi. Several multinucleated cells typical of syncytiotrophoblast were seen. The postoperative course was essentially uneventful. A postoperative pyelogram showed absence of the left kidney and collecting system.

Comment

Our case is unique because the heterotopic fallopian tube that contained the ectopic pregnancy was located at the pelvic brim and was associated with a unicornuate uterus. There also was absence of rudimentary horns and broad or round ligaments on the side of the heterotopic tube. Dabby and Nardone[1] reported a ruptured ectopic pregnancy in a fallopian tube found lateral to the ascending colon. The uterus was supposedly normal and the position of the ovary was not clear. Szlachter and Weiss[2] reported unilateral, segmental absence of a tube that contained an ectopic pregnancy associated with a bicornuate uterus.

This case illustrates interesting anatomic and physiologic features of the female genital tract. In addition, it demonstrates the phenomenon of transperitoneal migration of sperm. For pregnancy to have occurred in the heterotopic left fallopian tube, sperm must have traversed the uterus and the right tube and crossed the peritoneal cavity.

The association of uterus unicornis and agenesis of the contralateral kidney has been documented. In considering malformations of the female reproductive system one must appreciate their frequent association with defects of the urologic system. This relationship is easy to understand when one considers that embryologically the urinary and genital tracts have extremely close origins. At the sixth week of development the müllerian cleft appears between the lateral and the medial or gonadal portion of the urogenital ridge. The subsequent development of the müllerian system is closely related to the wolffian system. Major urinary tract anomalies have been found in a significant number of patients with imperfect müllerian duct development and vice versa. The importance of evaluating the urinary tract when clinicians encounter müllerian anomalies is emphasized.

A hysterogram should be obtained in these patients to identify any intrauterine anomalies. Hysterography was not performed in this patient because she conceived

before further evaluation. This pregnancy, however, recently ended with an intrauterine fetal death of unknown cause at 30 weeks' gestation.

In summary, this case demonstrates an unusual congenital anomaly consisting of a unicornuate uterus with a heterotopic left fallopian tube and a normal right tube and ovary. The left tube was not connected to the uterus and was attached to the left ovary by the fimbria ovarica. This tube contained an unruptured ectopic pregnancy, which provides supportive evidence for the phenomenon of transperitoneal sperm migration. The left kidney and collecting system failed to develop, emphasizing the importance of evaluating the urinary tract when müllerian anomalies are encountered.

REFERENCES

1. Dabby V, Nardone R. Ruptured ectopic pregnancy in an ectopic tube. J Fla Med Assoc 1977;64:809.
2. Szlachter N, Weiss G. Distal tubal pregnancy in a patient with a bicornuate uterus and segmental absence of the fallopian tube. Fertil Steril 1979;32:602.

Laparoscopy—A diagnostic aid in cases of suspected appendicitis

Its use in women of reproductive age

Nick M. Spirtos, M.D., Scott M. Eisenkop, M.D., Tanya W. Spirtos, M.D., Raymond I. Poliakin, M.D., and Lester T. Hibbard, M.D.

Los Angeles, California

In women of reproductive age the usefulness of laparoscopy in diagnosing acute appendicitis was evaluated. Eighty-six women underwent diagnostic laparoscopy. There was complete visualization of the appendix in 93% of the patients. Twenty-two patients were spared laparotomy. In the nonpregnant patients, salpingitis was the disease most often confused with appendicitis. Eighty-five percent of the patients with salpingitis had the onset of symptoms within 14 days of the last menstrual period, whereas acute appendicitis was found in 86% of the patients with the onset of symptoms >14 days after the last menstrual period. The onset of symptoms relative to the first day of the last menstrual period differed in these two groups of patients ($p < 0.01$). Patients who were spared unnecessary laparotomy had significantly diminished hospital stays ($p < 0.001$). Laparoscopy was found to be a safe and effective way to diagnose acute appendicitis in women of reproductive age, and its liberal use is recommended. (Am J Obstet Gynecol 1987;156:90-4.)

Key words: Pregnancy, ectopic, laparoscopy

Acute appendicitis is one of the few surgical diseases encountered in which a correct preoperative diagnosis is not mandatory. Negative laparotomy rates (the percentage of laparotomies resulting in the removal of a normal appendix) average 15% to 20% and are considered acceptable.[1-3] However, in women of reproductive age the negative laparotomy rate approaches 40%.[3-6] The differential diagnosis between inflammatory disorders of the female genital tract, appendicitis, torsion of adnexal structures, and other conditions remains a challenge. In addition, removal of a normal appendix is not without risk; complications include wound infection, pneumonia, peritonitis, pelvic abscess, paralytic ileus, bowel obstruction, deep vein thrombosis, infertility, and death.[7-10] Recent reports suggest that laparoscopy can significantly reduce the negative laparotomy rate associated with the diagnosis and treatment of acute appendicitis.[11, 12] This study reports the experience of the gynecology service at Los Angeles County–University of Southern California Medical Center in the use of laparoscopy for diagnosis in cases of suspected appendicitis.

From the Department of Obstetrics and Gynecology, University of Southern California School of Medicine, Women's Hospital, Los Angeles County–University of Southern California Medical Center.

Received for publication February 21, 1986; revised July 22, 1986; accepted August 14, 1986.

Reprint requests: Nick M. Spirtos, M.D., Department of Gynecology and Obstetrics, Stanford University School of Medicine, Stanford, CA 94305-5317.

Material and methods

Ninety-four women underwent laparoscopy to rule out appendicitis at Los Angeles County–University of Southern California Medical Center from 1974 to 1983. A two-puncture technique performed by resident and/or staff physicians was used. (Thirteen of the 94 patients were pregnant in the first trimester.) Eighty-six charts were reviewed for menstrual and contraceptive history as well as chief complaints, physical findings, and laboratory analysis. Eight charts were unavailable for review. All microscopic specimens were reviewed by one of us. (Statistical comparison was done with use of χ^2, Student's t test and Rankit analysis.)

Results

Eighty-six women between 16 and 42 years of age, with a median age of 21 years, presented with a chief complaint of abdominal pain. The differential diagnoses included appendicitis, salpingitis, pyelonephritis, torsion of the adnexa, ectopic pregnancy, and early intrauterine pregnancy complicated by acute appendicitis. Previously, because the diagnosis of appendicitis was considered, laparotomy would have been performed in all of these patients. Instead, laparoscopy was performed initially followed by laparotomy if indicated. Of the 86 patients, 80 had satisfactory laparoscopy (93%) in that visualization of the pelvic viscera and the entire appendix was possible.

Six laparoscopies were unsatisfactory; in these six patients the appendix could not be entirely visualized. In four instances the appendix could not be visualized because of the presence of pus and dense adhesions from ruptured appendices. In one instance the appendix could not be visualized because of the presence of a 200 ml spillage from a ruptured endometrioma. In the final instance the surgeon could not induce a pneumoperitoneum.

Of the 80 patients with satisfactory laparoscopy, 56 were thought to have acute appendicitis, 17 salpingitis, one a ruptured corpus luteum cyst, one a fecalith, and five were thought to have no pathologic condition present (Table I). Appendicitis was confirmed histologically in 51 patients; 47 correctly diagnosed at the time of laparoscopy and four diagnosed at the time of laparotomy following unsatisfactory laparoscopy. The nine patients whose diagnosis of appendicitis was incorrect at the time of laparoscopy were retrospectively diagnosed as having salpingitis, based on concurrent findings of both an inflammatory exudate on the fallopian tubes as well as the appendix and a histologic diagnosis of periappendicitis. Seventeen patients had a correct diagnosis of salpingitis at the time of laparoscopy as well as another 10 patients following laparotomy. These patients included the nine patients with salpingitis and concurrent periappendicitis and one patient who underwent laparotomy when the surgeon could not induce a pneumoperitoneum (Table I). Of the five patients who were thought to be disease-free at the time of laparoscopy, only one underwent laparotomy. Based on this patient's history, findings at exploratory laparotomy, and histologic review, a diagnosis of mesenteric adenitis was made. One patient had a ruptured corpus luteum cyst. Another patient was found to have a dilated appendix with multiple large fecaliths, and a final patient had a ruptured endometrioma.

Twenty-two patients underwent successful laparoscopy with visualization of the entire appendix and pelvic viscera, and laparotomy was avoided by confirmation of a nonsurgical diagnosis. Seventeen of these patients were found to have acute salpingitis. One patient was noted to have a ruptured corpus luteum cyst, and in four instances, no findings requiring laparotomy could be found at laparoscopy (Table I). All left the hospital with resolution of their physical findings and symptoms, and none later required surgical intervention.

The clinical presentations of the nonpregnant patients were remarkably similar. As in other series, salpingitis was the condition most often confused with

Table I. Laparoscopic and final diagnoses

	Laparoscopic diagnosis (n)	*Final diagnosis (n)*
Acute appendicitis	56	51
Salpingitis	17	27
Ruptured corpus luteum cyst	1	1
Mesenteric addenitis	0	1
Ruptured endometrium	1	1
Fecalith	1	1
No pathologic findings	5	4
Unsatisfactory	5*	0
Total	86	86

*Four patients had unsatisfactory laparoscopy with appendicitis; one patient had failed laparoscopy.

Table II. Presenting clinical data (nonpregnant patients)

	Patients with appendicitis	*Patients with salpingitis*
Median age (yr)	21	22
Range	16-42	(15-36)
Nausea and vomiting (%)	80	72
Anorexia (%)	89	85
Obstipation (%)	18	20
Temperature	100.6°	101°
Median range	98.0-103.2	98-102
White blood count	14,300	12,600
Range	(8,200-25,000)	(6,800-23,500)
Total no. of patients	51	27

Table III. Historic characteristics (nonpregnant patients)

	Patients with appendicitis	*Patients with salpingitis*
Menstrual pattern		
Normal	26	20
Abnormal	11	5
Total	37	25*
Contraception		
Oral contraceptives	6	6
Intrauterine device	6	5
Bilateral tubal ligation	1	1
None	24	12
	37	24

*Data not available on all patients.

Table IV. Onset of symptoms from last menstrual period (nonpregnant patients; n = 71)*

	≤14 days		*>14 days*	
	Appendicitis	*Salpingitis*	*Appendicitis*	*Salpingitis*
No. of patients	29	23	25	4

*Dates of last menstrual period not available for two patients.

appendicitis.[4,5] There was no remarkable physical finding, symptom, or laboratory test that proved to be of value in differentiating acute appendicitis from salpingitis. The percentage of patients in each group found to have a history of significant nausea and vomiting, anorexia, or obstipation for >24 hours was calculated (Table II). There was no significant difference between the salpingitis and appendicitis groups. The median age of the patients with appendicitis and salpingitis was 21 and 22 years old, respectively. The groups were remarkably similar in terms of menstrual patterns and contraception (Table III). Only when the onset of symptoms was compared to the first day of the last menstrual period did the two groups significantly differ; the patients with acute appendicitis were distributed equally throughout the menstrual cycle whereas most patients with salpingitis (85%) had their symptoms begin within 2 weeks of the last menstrual period ($p < 0.01$). Patients with the onset of symptoms at >14 days after the last menstrual period had acute appendicitis 86% of the time (Table IV).

Thirteen patients had positive pregnancy tests and were in their first trimester. In addition to menstrual histories significant for amenorrhea, the histories and physical findings of the pregnant patients in this series were similar to the nonpregnant patients. All 13 appendices were visualized; 12 were found to be acutely inflamed and one was normal. There were no maternal complications in this group and no fetal losses occurred.

Table V. Length of hospitalization

Procedure	*No. of patients*	*Hospital days*	*Significance*
Laparoscopy only	21	3.8 ± 2.5	$p < 0.001$
Laparoscopy-laparotomy with removal of normal appendix	14	5.7 ± 2.6	NS
Laparoscopy-laparotomy with removal of inflamed appendix	51	6.2 ± 3.3	

Table VI. Complications

Complications		
Type	*n*	*Final diagnosis*
Appendectomy		
Wound infections	4	Appendicitis (3); normal appendix (1)
Pelvic abscess	2	Appendicitis
Pulmonary embolism	1	Appendicitis
Ileus	1	Appendicitis
Laparoscopy		
None		

The patients with histologic confirmation of appendicitis had an average hospital stay of 6.2 ± 3.3 days (Table V). This is identical to the group of patients who underwent diagnostic laparoscopy and appendectomy with the removal of a normal appendix (5.7 ± 2.6 days). However, in those patients where laparoscopy prevented laparotomy, the average hospital stay was 3.8 ± 2.5 days, a significant decrease in hospitalization ($p < 0.001$). There were no complications among the patients spared laparotomy, but eight complications occurred in patients who required laparotomy (Table VI). It should be noted that seven of these eight patients did have acute appendicitis and needed laparotomy. There were no deaths in either group.

Comment

Recently three studies reported the use of laparoscopy in the diagnosis of acute appendicitis.[9,11,12] In 1979 the retrospective study of Leape and Ramenofsky[12] included 32 pediatric patients, the negative laparotomy rate was 12%, and six patients were spared laparotomy. There was one false negative laparoscopy. This patient developed increasingly severe peritoneal signs; appendectomy was performed and appendicitis was confirmed histologically. In 1982 Deutsch et al.[11] prospectively performed laparoscopy on 36 patients between 18 and 50 years of age. The negative laparotomy rate

was 4%, and 12 patients (33%) were spared laparotomy. There were no false-negative laparoscopies in this study. Leape and Deutsch were able to significantly reduce their negative laparotomy rates by using laparoscopy as a diagnostic aid.

In 1980 Jersky et al.[9] prospectively performed laparoscopy on 27 patients. After classification of patients into three groups (likely to have appendicitis, unlikely to have appendicitis, and uncertain), appendectomy was performed. Nineteen of the 27 patients were classified as unlikely to have appendicitis or had uncertain diagnoses. Twelve of these 19 patients had acute appendicitis, and thus Jersky et al. concluded laparoscopy would not improve their negative laparotomy rate. These results are so different from the above-mentioned studies, as well as our own, that further discussion is warranted. Although Jersky et al. classified patients as having an uncertain diagnosis if (1) the appendix was incompletely visualized (eight patients), (2) the appendix was normal and no other pathologic condition was identified (three patients), or (3) the appendix and other pelvic organs were concurrently inflamed (two patients), they did not state why the appendices were incompletely visualized or why patients with no evident pathologic condition were categorized into the uncertain group, and they did not describe what they considered coexistent salpingitis and appendicitis. Since this information is not provided, we feel it is not possible to evaluate Jersky et al.'s study and compare it to the results of others.

Unlike Jersky et al., who were unable to entirely visualize eight of 27 appendices (33%), we had little difficulty visualizing the appendix. Eighty of 86 patients (93%), including 13 pregnant patients, had appendices that were entirely visualized. Other than our one technical failure, all patients whose appendices could not be visualized ultimately proved to have surgical conditions; four had acute appendicitis, and one had a ruptured endometrioma. The diagnositc problems we encountered when the entire appendix was completely visualized were decisions regarding which organ was primarily affected when there was concurrent salpingitis and periappendicitis. In nine such patients, a review of the operative reports revealed that, in spite of the presence of gross pus exuding from inflamed fallopian tubes, a concerned surgeon decided to perform an appendectomy. The nine patients had only periappendicitis. Jersky et al. had two somewhat similar patients, and both of their patients had acute appendicitis. Thus it is somewhat difficult to wholeheartedly advocate abandoning appendectomy in such cases. In spite of the misdiagnosis in nine such cases, we were still able to halve our negative laparotomy rate by adding laparoscopy to our diagnostic armamentarium. In all, 22 patients were spared laparotomy. Perhaps as more data are gathered and more experience gained with use of laparoscopy as a diagnostic aid, laparotomy in similar cases will be avoided.

Another group of patients who presented special diagnostic problems were those presenting with lower abdominal pain, questionable uterine enlargement and a positive pregnancy test. Differential diagnoses included ectopic pregnancy, early intrauterine pregnancy with appendicitis or torsion of an adnexal structure, pyelonephritis, or a rupture of a corpus luteum cyst. Physical examinations in these patients were usually unproductive, since abdominal guarding often prevented an accurate assessment of uterine size and the adnexal structures. A correct diagnosis in this group of patients is critical because complications following a misdiagnosis include maternal and fetal death.[13, 14] Since we had only 13 pregnant patients in our study and all were in the first trimester, it is difficult to advocate laparoscopy in all pregnant patients. However, with the use of open laparoscopy, even patients with advanced gestations could undergo laparoscopy.

Other benefits of laparoscopy include decreased postoperative complications, shortened hospital stays, and a better selection of abdominal incisions. In the literature, 15% of patients whose normal appendix is removed by laparotomy develop significant postoperative complications such as pneumonia, intestinal obstruction, stress ulceration, ileus, peritonitis, pelvic abscess, deep vein thrombosis, wound infection, and death.[2, 7-9, 15] In addition, pregnant patients have an increased risk of spontaneous abortion. On the other hand, laparoscopy is associated with 1% to 2% postoperative complications.[7, 16] Laparoscopy reduces the length of hospital stay and provides the surgeon with information that can be used in selecting the location of the abdominal incision when laparotomy proves necessary. If acute appendicitis is diagnosed, a muscle-splitting incision can be made in the best location. A properly placed incision is particularly important in the pregnant patient in whom exposure can be especially troublesome.

Not only is laparoscopy in experienced hands usually safe and simple, but as a diagnostic procedure, it has proved to be quite valuable. In our series of 86 patients, the negative laparotomy rate was 19%; if laparoscopy had not been performed, the negative laparotomy rate would have been 41%. Therefore we recommend laparoscopy to rule out appendicitis in women of reproductive age. When the onset of symptoms is ≤14 days after the last menstrual period, laparotomy can often be avoided. With patients whose symptoms begin after 14 days from the last menstrual period, use of laparotomy, without laparoscopy, cannot be criticized. Since

some groups using laparoscopy have been able to decrease their negative laparotomy rate to as low as 5%, we still recommend laparoscopy in these patients. In the pregnant patient, laparoscopy also proved a valuable diagnostic aid, and we recommend its liberal use to further reduce negative laparotomy rates. Only when the entire appendix is visualized should laparoscopy be called satisfactory.

In summary, although these data were collected and analyzed retrospectively, valuable clinical information was acquired. First, it is apparent that, with experienced laparoscopists, adequate visualization of the appendix is possible in most patients (93%). In those patients where visualization was not adequate, significant pathologic conditions requiring laparotomy proved to be the reason visualization was not possible. Second, our institution's negative laparotomy rate was significantly reduced from 41% to 19% by use of laparoscopy. This study was not prospectively designed, but there are historic controls that have consistently documented negative laparotomy rates of 40% for women in the reproductive age group. This, as it happens, is almost identical to what ours would have been if laparoscopy had not been performed on our patients. Although there is some selection bias on our study population, it is difficult to conclude that we are overstating the benefit of laparoscopy in the differential diagnosis of appendicitis from salpingitis. Third, it is interesting to note that, as suspected, salpingitis develops much more commonly early in the menstrual cycle. In fact, only four of 29 (13%) of the patients with the onset of symptoms more than 14 days after the last menstrual period had salpingitis. With the above guidelines and use of the laparoscope, surgeons and obstetricians-gynecologists should be able to significantly reduce negative laparotomy rates in diagnosing women with suspected appendicitis.

REFERENCES

1. Cantrell JR, Stafford ES. The diminishing mortality from appendicitis. Ann Surg 1955;141:749.
2. Hobson T, Rosenman ID. Acute appendicitis—when is it right to be wrong? Am J Surg 1964;108:306.
3. Jess P, Bjerregard B, Brynitz S, et al. Acute appendicitis prospective trial concerning diagnostic accuracy and complications. Am J Surg 1981;141:232.
4. Dunn EL, Moore EE, Elerding SC, et al.: The unnecessary laparotomy for appendicitis—can it be decreased? Am Surg 1982;48:320.
5. Gilmore OJA, Browett JP, Griffin PH, et al. Appendicitis and mimicking conditions. Lancet 1975;2:421.
6. Silberman VA. Appendectomy in large metropolitan hospital. Am J Surg 1981;142:615.
7. Chamberlain G. Gynaecological laparoscopy. Ann R Coll Surg Engl 1980;62:133.
8. Howie JGR. Death from appendicitis and appendectomy—an epidemiological survey. Lancet 1966;4:72.
9. Jersky J, Hoffman J, Kurgan A. Laparoscopy in patients with suspected acute appendicitis. South African J Surg 1980;19:147.
10. O'Rourke M, Milton GW. The results of removing a "normal" appendix. Aust NZ J Surg 1963;33:12.
11. Deutsch AA, Zelikovsky A, Reiss R. Laparoscopy in the prevention of unnecessary appendectomies: A prospective study. Br J Surg 1982;69:336.
12. Leape LL, Ramenofsky ML. Laparoscopy for questionable appendicitis. Ann Surg 1979;191:410.
13. Babakni A, Hossein P, Woodruff JD. Appendicitis during pregnancy. Obstet Gynecol 1977;50:40.
14. Fisenda R, Roty AR, Kilway JR, et al. Acute appendicitis during pregnancy. Am Surg 1979;45:503.
15. Jerman RP. Removal of the normal appendix: the cause of serious complications. Br J Clin Pract 1969;23:466.
16. Chang FC, Hogle HH, Welling DR. The fate of the negative appendix. Am J Surg 1973;126:752.

cooperation with the University of Nevada,
hool of Medicine, Dept. of Obstetrics & Gynecology

GYN CME FUND
GYNECOLOGY CONTINUING MEDICAL EDUCATION
Las Vegas, Nevada

For 1987. . .
Three **weekend** programs designed to update and maximize **your** Continuing Professional Development!

- ☐ **Outstanding Faculty**. . . acknowledged authorities in their fields
- ☐ **Registration is easy**. . . use Visa, MasterCard and/or our convenient 800 number **1-800-NEV-OBGY — $375.00 for physicians and $200.00 for nurses**
- ☐ **Economical**. . . Take advantage of our three seminar package. Pay full registration fee for any two 1987 seminars and get the third one ***FREE.***
- ☐ **Relaxed atmosphere**. . . at the lovely Frontier Hotel on the Las Vegas Strip, adjacent to the fabulous Fashion Show Mall
- ☐ **"Take home messages"**. . . in syllabus provided at each seminar
- ☐ **Time available for one-on-one**. . . There's plenty of time for participant/faculty interaction!
- ☐ **Convenient Times!**. . . Two half-day sessions on a weekend. Minimizes time away from your practice. No Marathon sessions!
- ☐ **Money Back Guarantee**. . . if not satisfied

COMMON PROBLEMS IN AMBULATORY GYNECOLOGY, FEB. 7 and 8, 1987

lighlights: Transdermal Estrogens - a new approach to Menopause; Vulva/Vaginal Diseases - Where are they oing?; Periodic Screening for the GYN patient; Office Management for Urologic Problems; Outpatient Laser urgery for Infertility, & more!

aculty: William Droegemueller, M.D., Department of OB/GYN University of North Carolina; Thomas Lebherz, 1.D., Department of OB/GYN, UCLA School of Medicine; William Keye, Jr., M.D., Department of OB/GYN, Iniversity of Utah School of Medicine

REPRODUCTIVE ENDOCRINOLOGY IN THE OFFICE, SEPT. 26 and 27, 1987

lighlights: Update in Contraception; Amenorrhea and Athletics; PMS; Ovulation Induction; & more!

aculty: Daniel Mishell, M.D., Professor and Chairman, Department of OB/GYN, University of Southern Califor-ia; Richard Worley, M.D., Associate Professor and Associate Chairman, Department OB/GYN, University of Jtah, School of Medicine; Isaac Schiff, M.D., Associate Professor of Obstetrics and Gynecology, Department f OB/GYN, Brigham and Women's Hospital, Harvard Medical School.

CURRENT PROBLEMS IN OBSTETRICS, NOVEMBER 14 and 15, 1987

lighlights: Antenatal Diagnosis & Treatment 1987; The Cord Blood Gas, Your Friend in Court; Pregnancy and nvironmental Teratogens; Hepatitis in Pregnancy; and more!

aculty: Martin Pernoll, M.D., Chairman, Department of OB/GYN, Tulane University; Paul Summers, M.D., ssistant Professor, Department of OB/GYN, Tulane University

EGISTER NOW! The two-day seminars are $375.00 for physicians and $200.00 for nurses. Take advantage of our three seminar ackage — Pay full registration fee for any two 1987 seminars and get the third one ***FREE.*** The fee includes pen and syllabus material ach seminar), and breakfast buffet served at 7:00 a.m. each day. Refund Policy: Refund of registration fee, excluding a $25.00 ad-inistration fee will be made for cancellations made 10 days prior each seminar.

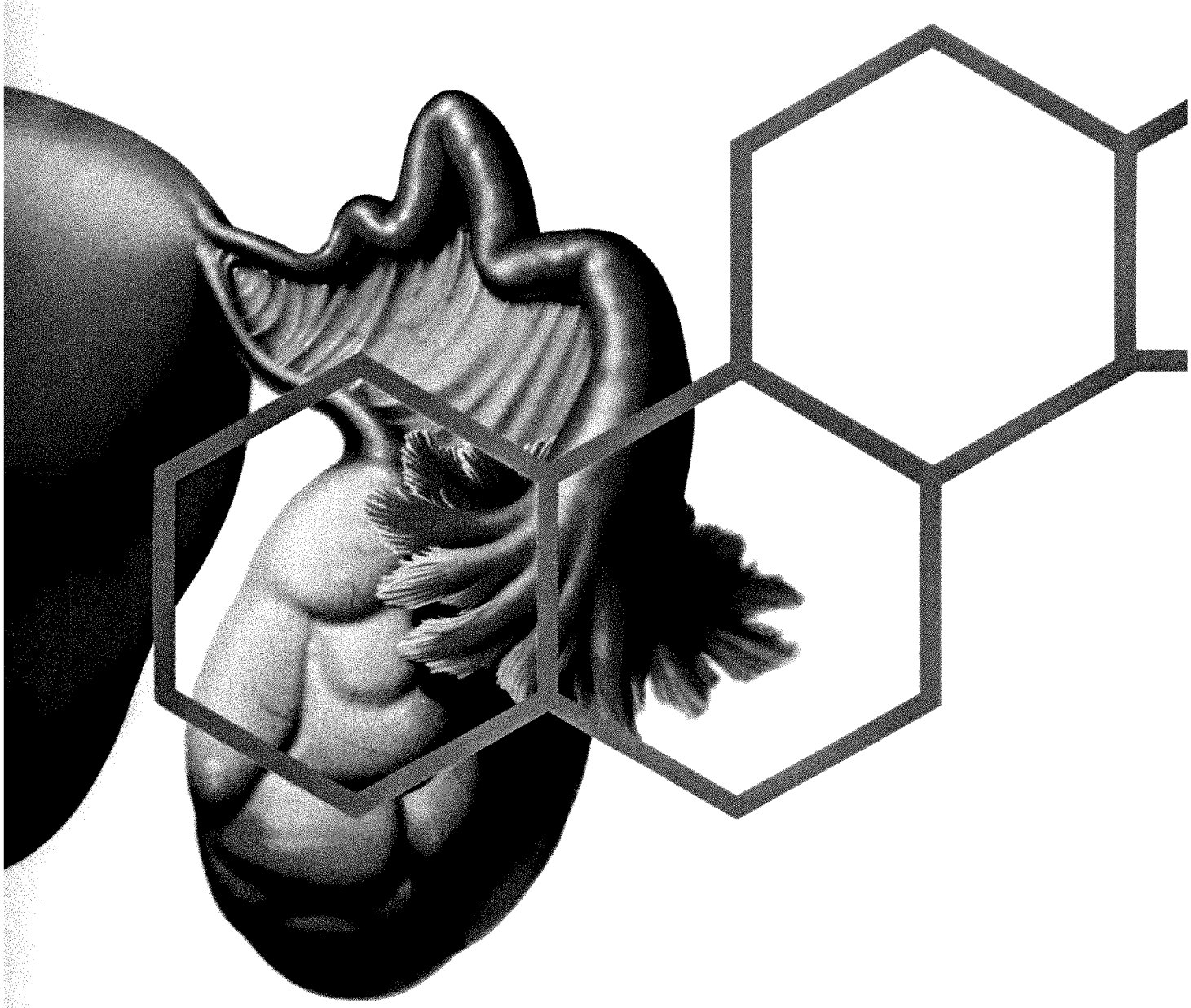
For Seminar Information Write or Call:

GYN CME FUND, 2570 S. Bruce Street, Suite B-4
Las Vegas, Nevada 89109-9957, 1-800-NEV-OBGY

FRONTIER HOTEL

THE RIGHT CHEMISTRY

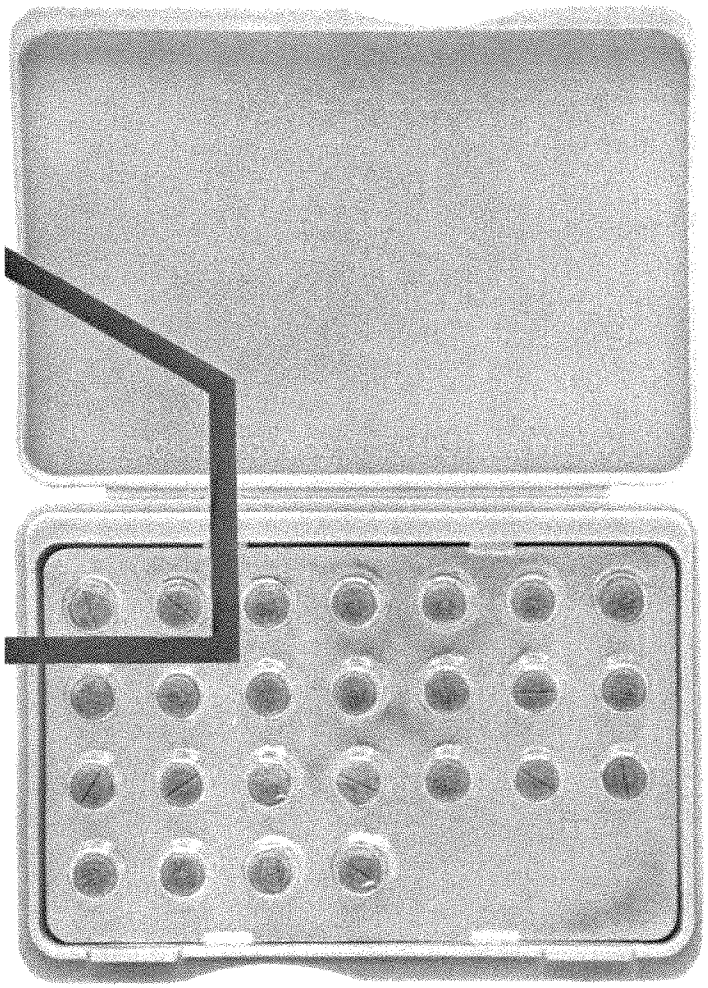
THE RIGHT REPLACEMENT FOR 17β-ESTRADIOL— 17β-ESTRADIOL



© 1986 Mead Johnson & Company • Evansville, Indiana 47721 USA MJL 5-4169

THE RIGHT REGIMEN

THE FIRST 25 DAYS OF THE MONTH— EVERY MONTH



ESTRACE is 17β-estradiol—just like the estrogen she made before menopause. Like the natural hormone, it follows a simple, well-known metabolic pathway, and it provides unsurpassed relief of postmenopausal vasomotor symptoms.

Micronized for good oral absorption, ESTRACE produces prompt, predictable serum levels of estradiol and estrone. Between cycles, ESTRACE is rapidly eliminated, providing estrogen replacement therapy that closely follows the premenopausal pattern . . . *for true cyclic therapy.*

Now it's in the ESTRACE Cycle Pak—an easy-to-carry compact, designed to deliver a starting dose of 2 mg in the preferred 25-day regimen. Cycle Paks delivering 1 mg daily are also available, if needed.

Mead Johnson LABORATORIES

For Brief Summary, please see following page.

ONLY NEW ESTRACE® (ESTRADIOL) TABLETS 1 mg and 2 mg CYCLE PAK HAS BOTH

ESTRACE® (estradiol)

TABLETS, 1 mg and 2 mg VAGINAL CREAM, 0.01%

Warning

1. **ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.** Three independent case control studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade. The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. **ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.** The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1000 exposures. Furthermore, a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb reduction defects. One case control study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed *in utero* to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. If ESTRACE® (estradiol) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation.

Indications: *Tablets:* Moderate to severe *vasomotor* symptoms associated with the menopause (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.); atrophic vaginitis; kraurosis vulvae; female hypogonadism; female castration; primary ovarian failure; breast cancer (for palliation only) in appropriately selected women and men with metastatic disease; prostatic carcinoma—palliative therapy of advanced disease. *Vaginal Cream:* Atrophic vaginitis and kraurosis vulvae. ESTRACE® HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

Contraindications: Known or suspected cancer of the breast, except in appropriately selected patients being treated for metastatic disease; known or suspected estrogen-dependent neoplasia; known or suspected pregnancy (See Boxed Warning); undiagnosed abnormal genital bleeding; active thrombophlebitis or thromboembolic disorders; a past history of thrombophlebitis, thrombosis or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

Warnings: Estrogens increase the risk of carcinoma of the endometrium (See Boxed Warning). Prescribe cautiously in women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms. A 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease has been reported in women receiving postmenopausal estrogens. There is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders or (except in treatment of malignancy) with a history of such disorders in association with estrogen use; they should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed. Large doses (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis; when estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptives should be considered a clear risk. Hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Increased blood pressure occurs with use of estrogens in the menopause, and blood pressure should be monitored, especially with high doses. Diabetic patients should be carefully observed for decreased glucose tolerance. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases; if this occurs, the drug should be stopped and appropriate measures taken to reduce the serum calcium level.

Precautions: *General*—A complete medical and family history should be taken prior to initiation of therapy. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination. Conditions influenced by fluid retention, such as epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc, may develop. Patients with a history of depression should be carefully observed. Pre-existing uterine leiomyomata may increase in size during estrogen use. Pathologists should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops, discontinue use while cause is investigated. Administer with caution in patients with impaired liver function, metabolic bone diseases associated with hypercalcemia, or renal insufficiency and in young patients with incomplete bone growth. The following endocrine and liver function tests may be affected by larger estrogen doses: increased sulfobromophthalein retention; increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability; increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay; free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered; impaired glucose tolerance; decreased pregnanediol excretion; reduced response to metyrapone test; reduced serum folate concentration; increased serum triglyceride and phospholipid concentration. *Information for the Patient*—See text of Patient Package Insert. *Pregnancy Category X*—See Contraindications and Boxed Warning. *Nursing Mothers*—Administer to nursing mothers only when clearly necessary.

The 2-mg tablets contain FD&C Yellow No. 5 (tartrazine), which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals, frequently those who also have aspirin hypersensitivity.

Adverse Reactions: (See Warnings regarding induction of neoplasia, adverse effects on the fetus, increased incidence of gall bladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.) The following additional adverse reactions have been reported: *Genitourinary system*—Breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea; premenstrual-like syndrome; amenorrhea during and after treatment; increase in size of uterine fibromyomata; vaginal candidiasis; change in cervical eversion and in degree of cervical secretion; cystitis-like syndrome. *Breast*—Tenderness, enlargement, secretion. *Gastrointestinal*—Nausea, vomiting; abdominal cramps, bloating; cholestatic jaundice. *Skin*—Chloasma or melasma which may persist when drug is discontinued; erythema multiforme, erythema nodosum, hemorrhagic eruption, loss of scalp hair, hirsutism. *Eyes*—Steepening of corneal curvature; intolerance to contact lenses. *CNS*—Headache, migraine, dizziness; mental depression; chorea. *Miscellaneous*—Increase or decrease in weight, reduced carbohydrate tolerance, aggravation of porphyria, edema, changes in libido.

How Supplied: Tablets, containing 1 mg (lavender) and 2 mg (turquoise) micronized estradiol, in bottles of 100 and compacts of 25; Vaginal Cream, each gram containing 0.1 mg 17β-estradiol in a nonliquefying base containing purified water, propylene glycol, stearyl alcohol, white ceresin wax, glyceryl monostearate, hydroxypropyl methylcellulose, sodium lauryl sulfate, methylparaben, disodium edetate, and *t*-butylhydroquinone, in 1½-oz (42.5-g) tubes with calibrated plastic applicator for delivery of 1,2,3, or 4 g and in 1½-oz (42.5-g) refill tubes.

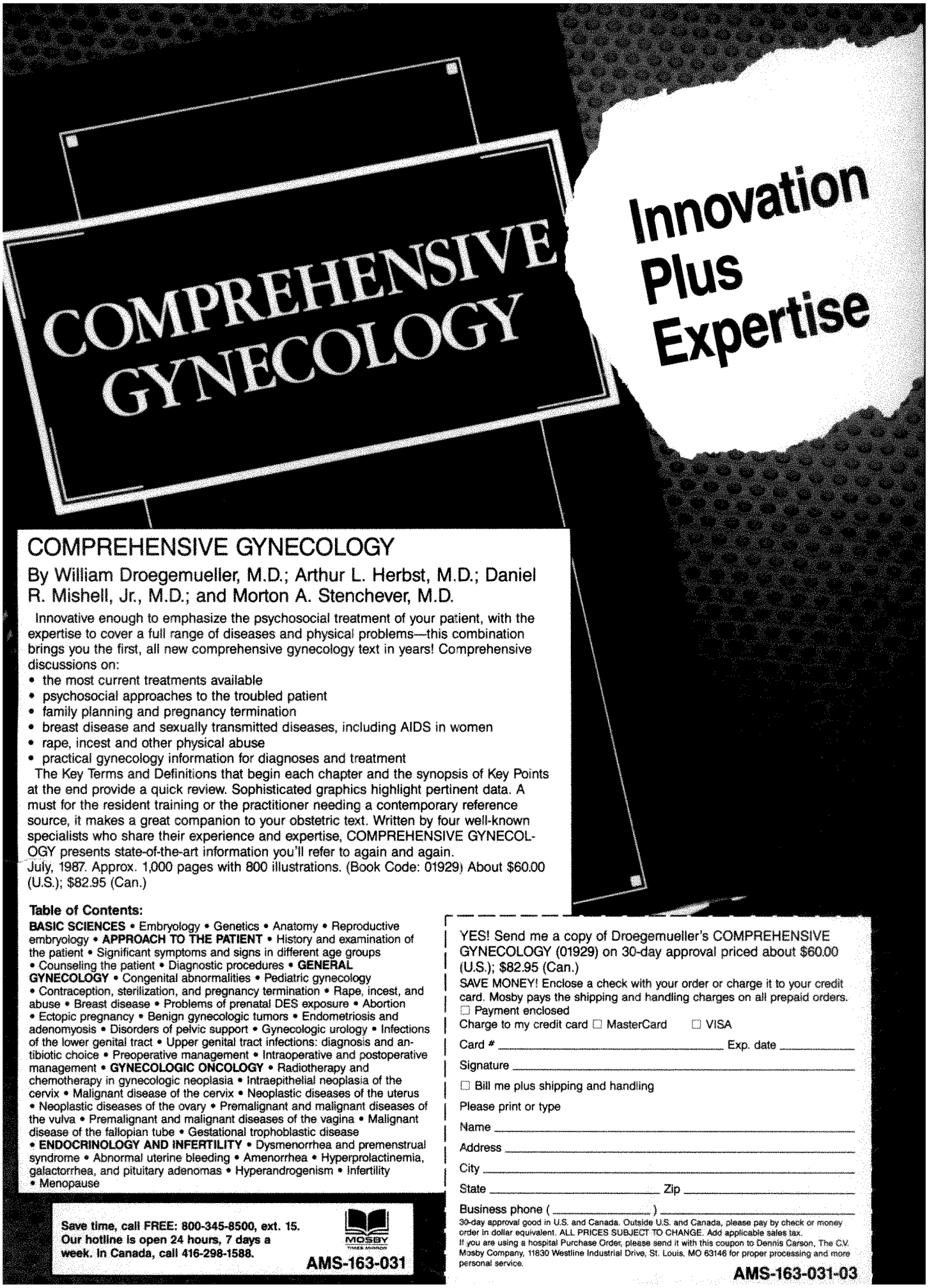
For Prescribing Information, please see physician package insert.

Mead Johnson LABORATORIES

MEAD JOHNSON & COMPANY • EVANSVILLE, INDIANA 47721 USA

MJL 5-4169

C-V14-12-85



COMPREHENSIVE GYNECOLOGY

By William Droegemueller, M.D.; Arthur L. Herbst, M.D.; Daniel R. Mishell, Jr., M.D.; and Morton A. Stenchever, M.D.

Innovative enough to emphasize the psychosocial treatment of your patient, with the expertise to cover a full range of diseases and physical problems—this combination brings you the first, all new comprehensive gynecology text in years! Comprehensive discussions on:

- the most current treatments available
- psychosocial approaches to the troubled patient
- family planning and pregnancy termination
- breast disease and sexually transmitted diseases, including AIDS in women
- rape, incest and other physical abuse
- practical gynecology information for diagnoses and treatment

The Key Terms and Definitions that begin each chapter and the synopsis of Key Points at the end provide a quick review. Sophisticated graphics highlight pertinent data. A must for the resident training or the practitioner needing a contemporary reference source, it makes a great companion to your obstetric text. Written by four well-known specialists who share their experience and expertise, COMPREHENSIVE GYNECOLOGY presents state-of-the-art information you'll refer to again and again.

July, 1987. Approx. 1,000 pages with 800 illustrations. (Book Code: 01929) About $60.00 (U.S.); $82.95 (Can.)

Table of Contents:

BASIC SCIENCES • Embryology • Genetics • Anatomy • Reproductive embryology • **APPROACH TO THE PATIENT** • History and examination of the patient • Significant symptoms and signs in different age groups • Counseling the patient • Diagnostic procedures • **GENERAL GYNECOLOGY** • Congenital abnormalities • Pediatric gynecology • Contraception, sterilization, and pregnancy termination • Rape, incest, and abuse • Breast disease • Problems of prenatal DES exposure • Abortion • Ectopic pregnancy • Benign gynecologic tumors • Endometriosis and adenomyosis • Disorders of pelvic support • Gynecologic urology • Infections of the lower genital tract • Upper genital tract infections: diagnosis and antibiotic choice • Preoperative management • Intraoperative and postoperative management • **GYNECOLOGIC ONCOLOGY** • Radiotherapy and chemotherapy in gynecologic neoplasia • Intraepithelial neoplasia of the cervix • Malignant disease of the cervix • Neoplastic diseases of the uterus • Neoplastic diseases of the ovary • Premalignant and malignant diseases of the vulva • Premalignant and malignant diseases of the vagina • Malignant disease of the fallopian tube • Gestational trophoblastic disease • **ENDOCRINOLOGY AND INFERTILITY** • Dysmenorrhea and premenstrual syndrome • Abnormal uterine bleeding • Amenorrhea • Hyperprolactinemia, galactorrhea, and pituitary adenomas • Hyperandrogenism • Infertility • Menopause

Save time, call FREE: 800-345-8500, ext. 15. Our hotline is open 24 hours, 7 days a week. In Canada, call 416-298-1588.

MOSBY
TIMES MIRROR

AMS-163-031

YES! Send me a copy of Droegemueller's COMPREHENSIVE GYNECOLOGY (01929) on 30-day approval priced about $60.00 (U.S.); $82.95 (Can.)

SAVE MONEY! Enclose a check with your order or charge it to your credit card. Mosby pays the shipping and handling charges on all prepaid orders.

☐ Payment enclosed

Charge to my credit card ☐ MasterCard ☐ VISA

Card # _____ Exp. date _____

Signature _____

☐ Bill me plus shipping and handling

Please print or type

Name _____

Address _____

City _____

State _____ Zip _____

Business phone (_____) _____

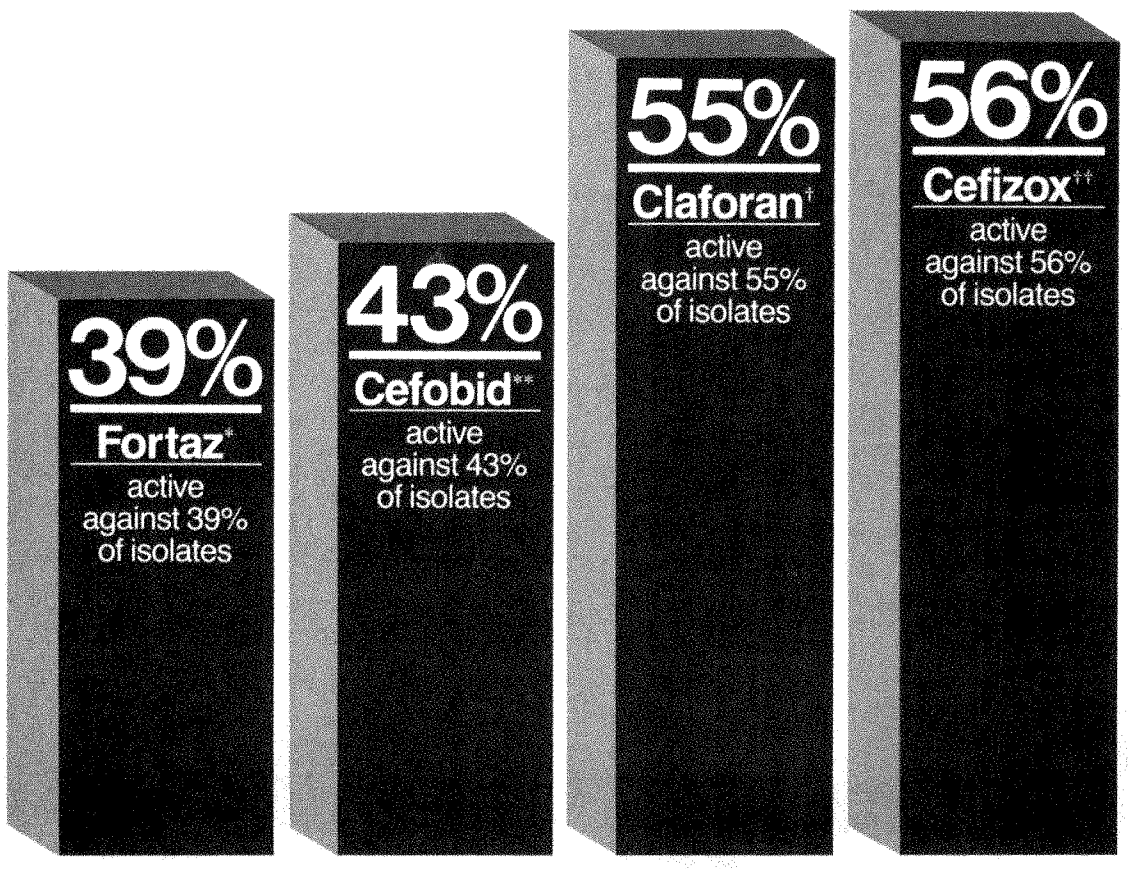
30-day approval good in U.S. and Canada. Outside U.S. and Canada, please pay by check or money order in dollar equivalent. ALL PRICES SUBJECT TO CHANGE. Add applicable sales tax.

If you are using a hospital Purchase Order, please send it with this coupon to Dennis Carson, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, MO 63146 for proper processing and more personal service.

AMS-163-031-03

Why settle for less activit

Don't Compromise



Copyright © 1987 by Merck & Co., Inc

*Fortaz is a registered trademark of Glaxo Inc.
**Cefobid is a registered trademark of Pfizer Inc.
†Claforan is a registered trademark of Roussel-Uclaf.
††Cefizox is a registered trademark of Fujisawa SmithKline Corporation.

gainst the *B. fragilis* group?

94%

Mefoxin

active against 94% of isolates

the facts *still* favor Mefoxin® IV/IM (Cefoxitin Sodium | MSD)

The *Bacteroides fragilis* group includes *B. fragilis, B. distasonis, B. ovatus, B. thetaiotaomicron, B. vulgatus.*

The percentages displayed are a weighted average of the susceptibilities reported for these organisms at an MIC breakpoint of 16 mcg/mL by the Antibiotic MIC Susceptibility Reports, National Summary, in *The Bacteriologic Report: Volume I 1986,* Totowa, NJ, BAC-DATA® Medical Information Systems, Inc. This average was weighted by the frequency of isolation of each organism.

NOTE: *In vitro* activity does not necessarily imply *in vivo* effectiveness.

MEFOXIN is contraindicated in patients who have shown hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

MEFOXIN is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Streptococcus faecalis*) and many strains of *Enterobacter cloacae.* Methicillin-resistant staphylococci are almost uniformly resistant to MEFOXIN.

For a Brief Summary of Prescribing Information, please see following page.

MSD
MERCK SHARP & DOHME

Mefoxin® IV/IM (Cefoxitin Sodium | MSD)

Indications and Usage: *Treatment*—Serious infections caused by susceptible strains of the designated microorganisms in the following diseases:

LOWER RESPIRATORY TRACT INFECTIONS, including pneumonia and lung abscess, caused by *Streptococcus pneumoniae* (formerly *Diplococcus pneumoniae*), other streptococci (excluding enterococci, e.g., *Strep. faecalis*), *Staphylococcus aureus* (penicillinase and non-penicillinase producing), *Escherichia coli*, *Klebsiella* species, *Hemophilus influenzae*, and *Bacteroides* species.

GENITOURINARY INFECTIONS. Urinary tract infections caused by *E. coli*, *Klebsiella* species, *Proteus mirabilis*, indole-positive *Proteus* (i.e., *P. morganii*, *P. rettgeri*, and *P. vulgaris*), and *Providencia* species. Uncomplicated gonorrhea due to *Neisseria gonorrhoeae* (penicillinase and non-penicillinase producing).

INTRA-ABDOMINAL INFECTIONS, including peritonitis and intra-abdominal abscess, caused by *E. coli*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group § and *Clostridium* species.

GYNECOLOGICAL INFECTIONS, including endometritis, pelvic cellulitis, and pelvic inflammatory disease, caused by *E. coli*, *N. gonorrhoeae* (penicillinase and non-penicillinase producing), *Bacteroides* species including the *B. fragilis* group §, *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and group B streptococci.

SEPTICEMIA caused by *Strep. pneumoniae* (formerly *D. pneumoniae*), *Staph. aureus* (penicillinase and non-penicillinase producing), *E. coli*, *Klebsiella* species, and *Bacteroides* species including the *B. fragilis* group §.

BONE AND JOINT INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing).

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis*, streptococci (excluding enterococci, e.g., *Strep faecalis*), *E. coli*, *P. mirabilis*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae*. Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.

Contraindications: Previous hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

Warnings: BEFORE THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOXITIN, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. GIVE WITH CAUTION TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. IF AN ALLERGIC REACTION TO CEFOXITIN OCCURS, DISCONTINUE THE DRUG. SERIOUS HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

Pseudomembranous colitis, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use. Broad-spectrum antibiotics alter normal flora of colon and may permit overgrowth of clostridia, a toxin produced by *Clostridium difficile* is a primary cause of antibiotic-associated colitis. Mild cases may respond to drug discontinuance alone. In more severe cases, management may include sigmoidoscopy, appropriate bacteriological studies, fluid, electrolyte and protein supplementation, and use of a drug such as oral vancomycin; isolation of the patient may be advisable. Other causes of colitis should also be considered.

Precautions: *General*—Total daily dose should be reduced in patients with reduced urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur from usual doses. Prescribe with caution in patients with a history of gastrointestinal disease, particularly colitis. Prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures.

Drug Interactions—Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.

Drug/Laboratory Test Interactions—High concentrations (> 100 mcg/mL) may interfere with measurement of serum and urine creatinine levels by the Jaffe reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. High concentrations may interfere with measurement of urinary 17-hydroxy-corticosteroids by the Porter-Silber reaction and produce false increases of modest degree in levels reported. A false-positive reaction for glucose in urine has been observed with CLINITEST ® reagent tablets.

Carcinogenesis, Mutagenesis, Fertility Impairment—No long-term animal study has been performed on carcinogenic or mutagenic potential. Rat studies at approximately three times maximum recommended human dosage revealed no effects on fertility or mating ability.

Pregnancy Category B—Reproduction studies in rats and mice did not reveal teratogenic or fetal toxic effects, although fetal weights were slightly decreased. In rabbits, cefoxitin was associated with a high incidence of abortion and maternal death, neither considered teratogenic. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers—Excreted in human milk. Exercise caution.

Pediatric Use—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.

Adverse Reactions: The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently. *Local Reactions*—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. *Allergic Reactions*—Rash (including exfoliative dermatitis), pruritus, eosinophilia, fever, and other allergic reactions including anaphylaxis. *Cardiovascular*—Hypotension. *Gastrointestinal*—Diarrhea, including documented pseudomembranous colitis during or after treatment, and, rarely, nausea and vomiting. *Blood*—Eosinophilia, leukopenia including granulocytopenia, neutropenia, anemia, including hemolytic anemia, thrombocytopenia, and bone marrow depression. A positive direct Coombs test may develop in some individuals, especially those with azotemia. *Liver Function*—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase. *Renal Function*—Elevations in serum creatinine and/or blood urea nitrogen levels and, rarely, acute renal failure.

Note: In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

How Supplied: Sterile cefoxitin sodium in vials and infusion bottles containing 1 gram or 2 grams cefoxitin equivalent and in 10-gram bulk bottles.

§ *B. fragilis*, *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, *B. vulgatus*

® Registered trademark of Ames Company, Division of Miles Laboratories, Inc.

For more detailed information, consult your MSD Representative or see Prescribing Information. Merck Sharp & Dohme, Division of Merck & Co., Inc., West Point, PA 19486. J6MF49(120)

MSD
MERCK SHARP & DOHME

OBSTETRICIAN GYNECOLOGIST-IN-CHIEF

The Mary Imogene Bassett Hospital, a 189-bed, rural referral center, and major teaching affiliate of Columbia University, is seeking candidates for this key appointment. The chief is responsible for overall clinical and teaching programs in Obstetrics and Gynecology. The Service is providing approximately 500 deliveries and about 4300 obstetrical clinic visits yearly. Volume in Gynecology is about 290 admissions and 7800 clinic visits. The department has four attending physicians, rotating transitional residents, a certified nurse midwife, a physician's assistant, plus substantial nursing and office staffing.

Cooperstown, on scenic Lake Otsego about 60 miles west of Albany, enjoys unusual recreational opportunities, a strong school system, and an array of cultural and artistic activities. The village is known for its air of history, museums and resort facilities.

The medical staff is organized as a salaried group practice with full fringe benefits including malpractice coverage, generous free time, conference leave, and immediate annuity participation.

Please send curriculum vitae and correspondence to:

Harlan D. Alpern, M.D.
Search Committee Chairman
The Mary Imogene Bassett Hospital
Cooperstown, New York 13326
telephone (607) 547-3700

THE MARY IMOGENE
BASSETT HOSPITAL

Michael Reese Health Plan
A LARGE HOSPITAL-BASED HMO

has openings for the following full-time position:

Obstetrician Gynecologist

THE MICHAEL REESE HEALTH PLAN, a well-established and rapidly-expanding, federally-qualified HMO seeks Board Certified/Eligible Obstetrician/Gynecologists.

THIS POSITION entails both outpatient and hospital practice as well as medical students and house staff teaching.

REMUNERATION is high and competitive with academic and fee-for-service practice markets. Fringe benefits are generous.

INTERESTED APPLICANTS should send Curriculum Vitae in confidence to:
Dr. Stephen Contro, Medical Director, MICHAEL REESE HEALTH PLAN, 2545 Martin Luther King Drive, Chicago 60616

an equal opportunity employer m/f/h

Proliferation of group B streptococci in human amniotic fluid in vitro

Iffath A. Abbasi, Lieutenant Commander, MC, USNR,
Val G. Hemming, Colonel, MC, USAF, Gary S. Eglinton, Colonel, MC, USAF, and
Timothy R. B. Johnson, Major, MC, USAF
Bethesda, Maryland

The group B *Streptococcus* is one of the most virulent organisms causing perinatal infection. Human amniotic fluid from the second and third trimesters was pooled and analyzed for electrolytes, protein, albumin, zinc, inorganic phosphorus, ferritin, lysozyme, and immunoglobulins. We inoculated replicates of specimens with known virulent strains of group B streptococci (893, 891, and 878) and *Escherichia coli* (C_5) with Todd-Hewitt broth and normal saline solution used as controls. Group B streptococci strains 893 and 891 proliferated rapidly at rates similar to their rates in Todd-Hewitt Broth. Strain 878 grew at a rate slower than that of strains 893 and 891. The amniotic fluid specimens were similar with respect to factors reported as inhibitory to bacterial proliferation. Second- and third-trimester amniotic fluid supports the growth of group B streptococci as well as a culture medium optimized for bacterial growth. Strain-specific variance in group B streptococci growth rates in amniotic fluid may have clinical significance for those at risk for group B streptococci infection. (AM J OBSTET GYNECOL 1987;156:95-9.)

Key words: Group B streptococci, bacterial growth, amniotic fluid

Bacterial invasion and growth in amniotic fluid play an important role in infectious perinatal morbidity and mortality. Cattaneo[1] was the first to report the presence of antibacterial activity in amniotic fluid. Since then several investigators have identified a number of factors that have been reported to confer antibacterial properties to amniotic fluid. However, the presence of these factors does not conclusively explain the antibacterial activity.[2,3] Clinically, group B *Streptococcus* appears to be one of the most virulent organisms involved in perinatal infections. We analyzed human amniotic fluid for the various factors previously reported to alter its antibacterial activity and then plotted in vitro growth curves, during 24 hours, of known virulent strains of serotype III group B streptococci (strains 878, 891, and 893) in the amniotic fluid.

Material and methods

Amniotic fluid specimens were obtained, under aseptic conditions, by amniocenteses, performed either during the second trimester for fetal chromosomal analysis or during the third trimester for fetal lung maturation studies. All patients were free of infection and had received no medications other than prenatal vitamins or iron supplements. Blood- or meconium-stained fluid was excluded as was fluid yielding positive bacterial cultures. The specimens were centrifuged at 2000 rpm for 10 minutes at 10° C. Each specimen was filtered through a 0.45 μm filter to remove particulate matter. The fluid was then stored at −80° C until analysis.[4] All second-trimester amniotic fluid specimens were grouped into a single pool as were all third-trimester

Table I. Comparative analysis of the factors reported to alter the antibacterial properties of amniotic fluid

	Second-trimester pool	*Third-trimester pool*
Sodium (mEq/L)	134	124
Potassium (mEq/L)	3.8	3.7
Chloride (mEq/L)	112	107
Bicarbonate (mEq/L)	13.4	13.3
Glucose (mg/100 ml)	51	36
Calcium (mEq/L)	7.4	6.3
Protein (gm/100 ml)	0.4	0.4
Albumin (gm/100 ml)	0.3	0.2
Transferrin (mg/100 ml)	36	38.5
Zinc (mg/ml)	0.44	0.39
Inorganic phosphorus (mg/ml)	141	102
Zinc/inorganic phosphorus (mg/ml)	<200	<200
Lysozyme (mg/ml)	5.7	173
IgG	Not detected	Not detected

From the Department of Obstetrics and Gynecology, Naval Hospital, and the Departments of Obstetrics and Gynecology and Pediatrics, Uniformed Services University of the Health Sciences.
Supported in part by CO8606 from the Uniformed Services University of the Health Sciences.
Presented at the Sixth Annual Meeting of the Society of Perinatal Obstetricians, San Antonio, Texas, January 30–February 1, 1986.
The opinions expressed in this article are those of the authors and should not be construed as official or as reflecting the views of the United States Navy, United States Air Force, or the Department of Defense.
Received for publication July 16, 1986; accepted July 30, 1986.
Reprint requests: LCDR Iffath A. Abbasi, MC, USNR, Naval Hospital, Bethesda, MD 20814-5011.

Table II. In vitro proliferation of group B streptococci in second-trimester pooled amniotic fluid

Strain	Proliferation (CFU/ml)				
	0 hr	*2 hr*	*4 hr*	*6 hr*	*24 hr*
Group B streptococci					
878	2.4×10^4	1.8×10^4	3.6×10^4	1.8×10^5	5.0×10^5
891	5.7×10^4	5.8×10^5	1.0×10^6	6.4×10^7	2.4×10^8
893	6.2×10^3	1.7×10^4	3.3×10^5	7.0×10^6	6.0×10^7
E. coli (C_5)	2.9×10^4	2.5×10^4	5.5×10^5	1.7×10^6	2.2×10^6
Todd-Hewitt broth					
893	2.4×10^4	1.7×10^5	9.7×10^5	2.0×10^6	1.8×10^7
C_5	2.8×10^4	2.7×10^5	6.0×10^5	3.8×10^5	3.0×10^5
Normal saline solution					
893	1.6×10^4	1.7×10^4	1.1×10^4	5.1×10^3	1.6×10^3
C_5	1.2×10^4	1.0×10^4	1.1×10^4	1.6×10^4	1.3×10^4

Table III. In vitro proliferation of group B streptococci in third-trimester pooled amniotic fluid

Strain	Proliferation (CFU/ml)				
	0 hr	*2 hr*	*4 hr*	*6 hr*	*24 hr*
Group B streptococci					
878	1.8×10^4	2.6×10^4	3.0×10^4	3.2×10^5	4.8×10^5
891	5.2×10^4	5.8×10^5	9.7×10^5	4.0×10^6	4.8×10^7
893	6.0×10^4	1.9×10^5	1.6×10^6	4.0×10^7	4.2×10^8
E. coli (C_5)	2.9×10^4	2.7×10^5	5.3×10^5	4.1×10^6	1.7×10^6
Todd-Hewitt broth					
893	1.0×10^4	1.9×10^5	2.8×10^5	2.6×10^6	1.6×10^7
C_5	2.7×10^4	1.8×10^4	5.6×10^5	3.2×10^5	2.3×10^6
Normal saline solution					
893	1.3×10^4	1.8×10^4	2.6×10^4	1.2×10^4	1.0×10^4
C_5	1.6×10^4	1.7×10^4	1.7×10^4	5.1×10^3	1.6×10^3

specimens. Amniotic fluid from each pool was cultured to ensure sterility and analyzed for the factors previously reported to inhibit the bacterial growth in amniotic fluid. Measurements included the analysis of electrolytes, total proteins, albumin, zinc, inorganic phosphorus, transferrin, lysozyme activity, and immunoglobulin levels. Zinc levels were measured after wet digestion with nitric-sulfuric acid followed by measurement by atomic absorption at 214 nm in air-acetylene flame with the use of a Perkin-Elmer atomic absorption instrument (Model 603, Beckman Instruments, Inc., Fullerton, California). Extensive precautions were required to avoid contamination of the samples by atmospheric dust, a common source of traces of zinc. All glassware was washed with acid before use. Transferrin levels were quantitated by means of the single radial immunodiffusion technique previously described by Larsen et al.[5] Lysozyme activity was measured with a commercial assay kit (Sigma Chemical Company, St. Louis, Missouri).

To plot the bacterial growth curves, paired 2 ml aliquots of amniotic fluid were inoculated in duplicate at zero time with approximately 1×10^4 CFU/ml of known virulent strains of serotype III group B streptococci (878, 891, and 893) and with strain C_5 *Escherichia coli*. Inocula were prepared by growing the group B streptococci to log phase (6 hours) in Todd-Hewitt broth, then adjusting the concentration to 1×10^9, cfu/ml, washing in sterile nonbacteriostatic normal saline solution, and adjusting to a concentration of approximately 1×10^4 cfu/ml. Twenty microliters of the saline solution group B streptococci or *E. coli* suspensions was inoculated into each amniotic fluid specimen. The specimens were mixed well; aliquots were removed for quantitative cultures and incubation was begun at 37° C in room air. Aliquots of 2 ml of Todd-Hewitt broth and of normal saline solution were used as positive and negative controls, respectively.

Duplicate 10 μl aliquots were removed at 2, 4, 6, and 24 hours from each specimen. After careful mixing, quantitative cultures were performed by successive tenfold dilutions of each sample followed by inoculation of a measured volume of each dilution onto blood agar plates. After overnight incubation at 37° C, colony counts were performed. The resulting growth curves were analyzed.

Results

The electrolytes, glucose, calcium, and total protein levels were within the normal ranges and were similar

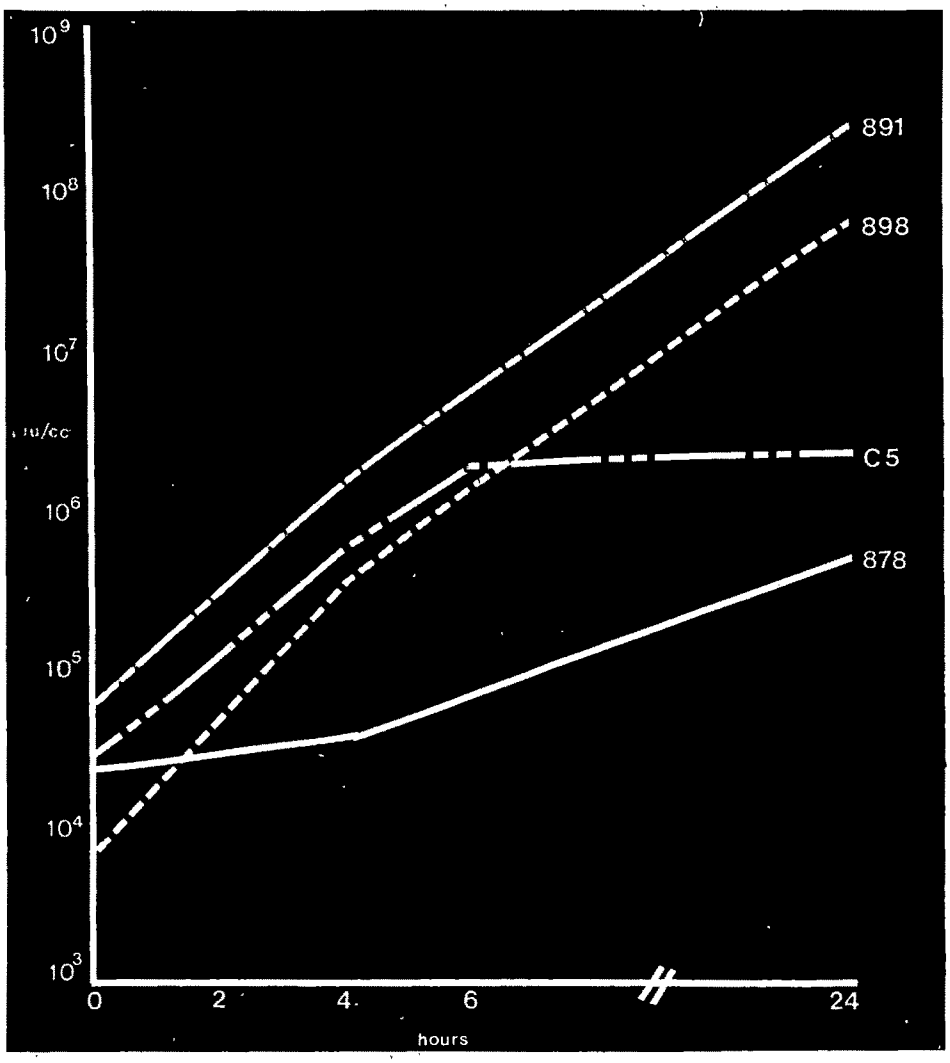


Fig. 1. Serotype III group B streptococci (strains 893, 891, and 878) and *E. coli* (strain C_5) growth curves during 24 hours in second-trimester human amniotic fluid.

in the two pools (Table I). The transferrin levels were similar to those previously reported for human amniotic fluid. Inorganic phosphorus values were also similar in the two pools, as were the zinc levels. The calculated ratio of inorganic phosphorus to zinc was >200. The lysozyme concentrations in the two pools were within the ranges previously reported for amniotic fluid. Immunoglobulin levels were not found in the amniotic fluid in amounts detectable by the radial immunodiffusion method.

Tables II and III show the results of the assays of the two pools during 24 hours for serotype III group B streptococci strains 878, 891, and 893, *E. coli* strain C_5 in amniotic fluid, Todd-Hewitt broth, and normal saline solution, and group B streptococci strain 893 in Todd-Hewitt broth and normal saline solution. All three strains of serotype III group B streptococci proliferated in amniotic fluid. The growth of 893 in amniotic fluid equaled or exceeded its growth in the positive control medium, Todd-Hewitt broth, whereas there was a log 1 decrease in colony-forming units per milliliter in the negative control, normal saline solution, which is not a nutrient medium.

Figs. 1 and 2 show a log-linear display of the growth curves for group B streptococci strains 893, 891, and 878 and *E. coli* C_5 in second- and third-trimester amniotic fluid. Strain 893 proliferated in both the second- and third-trimester pools at rates that exceeded its growth in Todd-Hewitt broth. Even though all three strains did proliferate in human amniotic fluid, they did so at different rates. Strain 878 did not match the apparently more aggressive strains 893 and 891. Fig. 2 shows the same growth curves in third-trimester amniotic fluid, thereby permitting a direct comparison of the three strains and once again demonstrating their different rates of growth in human amniotic fluid.

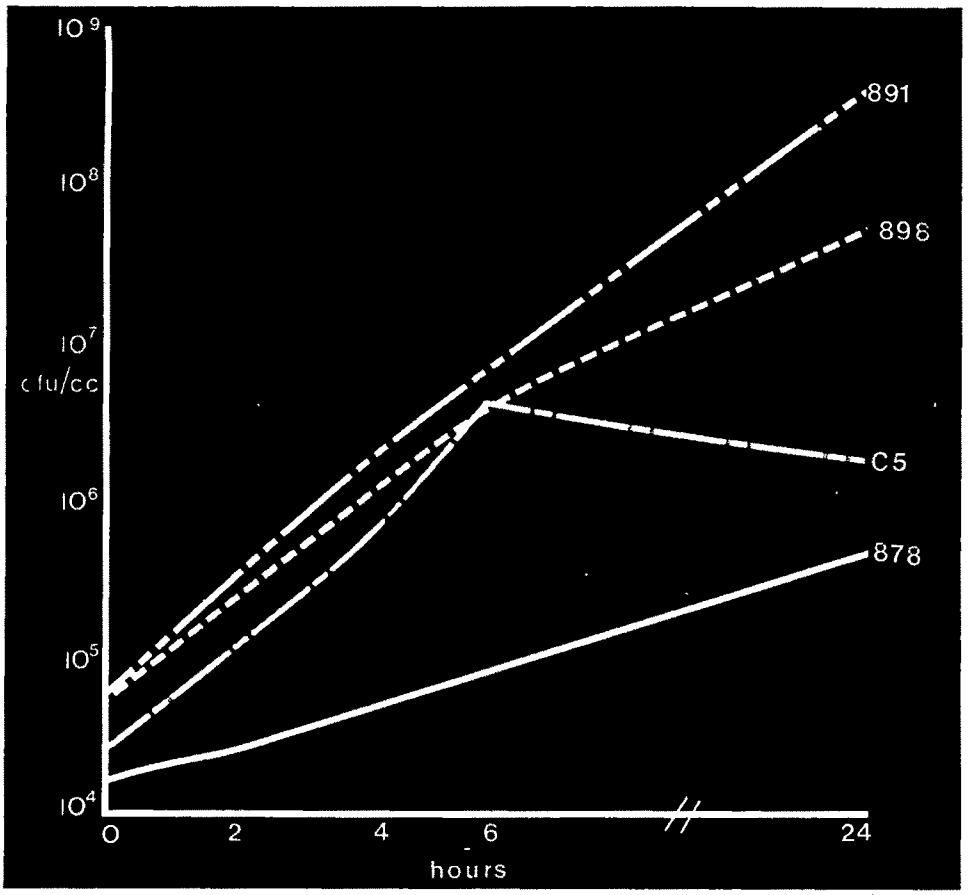


Fig. 2. Serotype III group B streptococci (strain 893, 891, and 878) and *E. coli* (strain C_5) growth curves during 24 hours in third-trimester human amniotic fluid.

Comment

Group B streptococci play an important role in bacterial infections of the mother and fetus. These organisms gain access to the amniotic fluid through ruptured or, more rarely, intact membranes and thereby present the fetus with a formidable bacterial challenge. While a substantial literature explores the various factors in amniotic fluid that impede bacterial growth, little is known about the influence of these factors on the prevention of the growth of group B streptococci. It has been reported that transferrin is capable of suppressing bacterial growth. Larsen et al.[5] have reported levels of transferrin concentrations in amniotic fluid with increasing gestational ages. These values correlate well with the levels found in our assays. Whether the zinc peptide complex inhibits proliferation of group B streptococci in amniotic fluid in vivo is uncertain. Schlievert et al.[6] isolated a zinc peptide complex that inhibits the proliferation of certain organisms in amniotic fluid. They demonstrated that ratios of inorganic phosphorus to zinc in amniotic fluid were predictive of the antibacterial activity of the fluid. A ratio of <100 was bactericidal, a ratio of 100 to 200 was bacteriostatic, and a ratio exceeding 200 was noninhibitory. However, these authors did not test this ratio against the growth of group B streptococci in amniotic fluid. In the present study, the group B streptococcal organisms proliferated to levels exceeding 1×10^7 CFU/ml in the presence of a ratio of inorganic phosphorus to zinc >200.

It has long been recognized that lysozymes play a role in host resistance. As early as 1949, Cattaneo[1] reported that amniotic fluid contained lysozyme-like activity. Since then, other authors have reported its presence and have quantified its levels in amniotic fluid.[7, 8] The levels obtained by our assays compared favorably with those reported by these authors. Since immunoglobulins represent an important host defense mechanism in the body, their levels have been quantified and reported for both normal and infected amniotic fluid. While immunoglobulin levels were not detected by our assay methods, lower levels cannot be excluded by this study because the lower limit of detection of the assay was 20 mg/100 ml.

Hemming et al.[4] reported the strikingly rapid proliferation of group B streptococci as compared with that

of *E. coli* in human amniotic fluid. In the present study, all three strains of group B streptococci proliferated rapidly during 24 hours, as did *E. coli* C_5. Our growth curves are similar to those reported by these and other authors.[9] However, there was an interstrain variation in the growth curves of group B streptococci. Even though serotype strain 878 of III group B streptococci did proliferate rapidly, as previously noted, it did so at a rate significantly lower than that of strains 893 and 891. Although growth curves of group B streptococci during 24 hours are not visually linear during the full 24-hour period, most curves are statistically linear when tested by linear regression analysis, which permits their comparison and allows demonstration of the differences in growth curves of the different strains in amniotic fluid. This variation may possibly be due to the accumulation, during 24 hours, of the metabolic end products of the bacteria that leads to a fall in pH and hence a deleterious effect on the growth and proliferation of these organisms, or it may be due to the consumption of nutrients that are essential to the growth of these organisms in the amniotic fluid.

Our preliminary data document strain-specific variation in growth rates of group B streptococci in human amniotic fluid in vitro. The identification of this variance may have important clinical significance for women and neonates at risk for group B streptococcal infection.

REFERENCES

1. Cattaneo P. Potere lisizimico del liquido amniotico postere antilisizimico del meconio, richere sperimentoli. Clin Ostet Ginecol 1949;51:60.
2. Courcol R, Roussel-Dellvallez J, Puech F. Quantitative bacteriological analysis of amniotic fluid. Biol Neonate 1982; 42:166.
3. Thadepalli H, Bach VT, Davidson EC. Antimicrobial effect of amniotic fluid. Obstet Gynecol 1978;52:198.
4. Hemming VG, Nagarajan K, Hess LW. Rapid in vitro replication of Group B Streptococcus in term human amniotic fluid. Gynecol Obstet Invest 1985;19:124.
5. Larsen B, Snyder IS, Galask RP. Transferrin concentration in human amniotic fluid. AM J OBSTET GYNECOL 1973; 117:952.
6. Schlievert P, Johnson W, Galask RP. Bacterial growth inhibition by amniotic fluid. V. Phosphate-to-zinc ratio as a predictor of bacterial growth-inhibitory action. AM J OBSTET GYNECOL 1976;125:899.
7. Larsen B, Galask RP, Snyder IS. Muramidase and peroxidase activity of human amniotic fluid. Obstet Gynecol 1974;44:219.
8. Cherry SH, Filler M, Harvie H. Lysozyme content of amniotic fluid. AM J OBSTET GYNECOL 1973;116:639.
9. Blanco JD, Gibbs RS, Krebs LF. The association between the absence of amniotic fluid bacterial inhibitory activity and intra-amniotic infection. AM J OBSTET GYNECOL 1982; 143:749.

Urinary diagnostic indices in preeclampsia-associated oliguria: Correlation with invasive hemodynamic monitoring

Wesley Lee, M.D., Bernard Gonik, M.D., and David B. Cotton, M.D.
Houston, Texas

Seven oliguric women with preeclampsia were identified among patients admitted for obstetric care at Jefferson Davis or Hermann Hospitals in Houston, Texas. Urinary diagnostic indices (including urine-to-plasma ratios of creatinine, urea nitrogen, and osmolality) were generally consistent with a prerenal etiology for the observed oliguria. Conversely, invasive hemodynamic monitoring revealed a volume replete state in five of seven preeclamptic women studied. All patients were observed to have markedly elevated urinary sodium concentrations. The transient oliguria observed in these patients spontanously resolved without diuretic or hyperosmolar agents. We conclude that oliguria is a poor index of volume status in preeclamptic women. Urinary diagnostic indices may also be misleading if used to guide fluid management in these patients. Finally, the clinical significance and therapeutic alternatives relating to preeclampsia-associated oliguria are discussed. (Am J Obstet Gynecol 1987;156:100-3.)

Key words: Oliguria, preeclampsia, pulmonary artery catheter

Urinary output is commonly regarded as a clinical index of volume status and renal function. In the presence of oliguria, urinary diagnostic indices have been used in nonpregnant patients to differentiate between prerenal and renal etiologies.[1] Oliguria is also a recognized peripartum complication of pregnancy-induced hypertension (PIH). However, a paucity of information regarding PIH-associated oliguria is presently available. Additionally, the validity of urinary diagnostic indices under these special circumstances has never been tested. An interinstitutional review recently identified seven preeclamptic women who developed oliguria and were followed by pulmonary artery catheterization. The present study examines PIH-associated oliguria with a indirect estimation of volume status, that is, the pulmonary capillary wedge pressure. Urinary diagnostic indices were simultaneously obtained to determine the clinical usefulness of this information relative to volume status as estimated by the hemodynamic monitoring.

Material and methods

The patient population was selected from preeclamptic women admitted to either Jefferson Davis Hospital or Hermann Hospital in Houston, Texas, during the period from February 1983 to December 1985. The diagnosis of preeclampsia included the criteria of newly onset gestational hypertension (>140/90 mm Hg) and proteinuria in the absence of preexisting renal disease. All patients received intravenous magnesium sulfate for seizure prophylaxis. A pulmonary artery catheter was percutaneously inserted under aseptic conditions for the determination of each patient's hemodynamic profile. Continuous monitoring of systemic blood pressure was provided by radial artery cannulation. Urine output was measured continuously with an indwelling Foley catheter. For the purposes of this study, oliguria was defined as urine output of <30 ml/hr for at least 2 consecutive hours. When oliguria was identified, blood and urine specimens were obtained for measurement of sodium, creatinine, urea nitrogen, and osmolality. Pulmonary capillary wedge pressure measurements were obtained during the oliguric phase. The pulmonary capillary wedge pressure was obtained with patients in the supine position supported by a wedge under the right hip. Intravenous fluid management was guided by hemodynamic parameters. No diuretics were employed during the study period. The blood and urine laboratory values were used to calculate urine-to-plasma ratios of sodium (U/P Na), creatinine (U/P Cr), urea nitrogen, and osmolality. Free excretion of sodium was calculated by the formula (U/P Na ÷ U/P Cr) × 100. The renal failure index was also calculated by dividing urinary sodium concentration (mEq/L) by (U/P Cr). The interpretation of urinary diagnostic indices was based on the criteria proposed by Miller et al.[1] (Table I). Data are reported as mean ± SD.

From the Divisions of Maternal-Fetal Medicine, Departments of Obstetrics and Gynecology, Baylor College of Medicine and University of Texas Health Sciences Center at Houston.
Presented in part at the Sixth Annual Meeting of The Society of Perinatal Obstetricians, San Antonio, Texas, January 30–February 1, 1986.
Received for publication January 31, 1986; revised July 7, 1986; accepted July 15, 1986.
Reprint requests: David B. Cotton, M.D., Department of Obstetrics and Gynecology, One Baylor Plaza, Houston, TX 77030.

Results

Demographic data from the seven study patients are tabulated in Table II. All but one patient presented before term with a mean gestational age of 34 ± 3 weeks. The mean arterial pressure for the group was 125 ± 16 mm Hg. Study subjects were primigravid women with the exception of patient No. 4 (gravida 2, para 1-0-0-1).

Table III summarizes pulmonary capillary wedge pressures and urinary diagnostic indices from each of these patients. The mean urine output was 23 ± 6 ml/hr. The mean pulmonary capillary wedge pressure was 8.7 ± 4.0 mm Hg. In only two patients (Nos. 3 and 7) was there evidence of intravascular hypovolemia as estimated by pulmonary capillary wedge pressure. The remainder suggested a volume replete state with normal to slightly elevated pulmonary capillary wedge pressure values.

Table IV allows classification of the urinary diagnostic indices by prerenal and intrarenal causes on the basis of criteria previously described (Table I). For most patients the urinary diagnostic indices could not be uniformly attributable to either prerenal or intrarenal factors. In this regard all patients demonstrated an elevated urine sodium consistent with a intrinsic renal defect. Conversely, most of the remaining indices supported a prerenal picture. An exception was found in patient No. 4 in whom the indices all were consistent with intrinsic renal disease.

In five of seven patients a volume replete state was suggested by normal to slightly elevated pulmonary capillary wedge pressure. Within this normovolemic group, one patient showed urinary diagnostic indices suggesting intrinsic renal failure, one revealed a mixed pattern, and the remaining three demonstrated a prerenal picture. Of note, the only patient (No. 6) with diagnostic indices clearly consistent with a prerenal picture had one of the highest pulmonary capillary wedge pressure in this study.

Comment

This study examines PIH-related oliguria and demonstrates a poor correlation between this clinical parameter and low pulmonary capillary wedge pressure. In this series of seven oliguric patients, only two subjects were considered hypovolemic by invasive hemodynamic monitoring. Urinary diagnostic indices also did not appear to be a useful indicator of volume status in these patients. The majority of indices predicted prerenal dehydration in five of seven preeclamptic women despite pulmonary capillary wedge pressures consistent with euvolemia.

Since oliguria associated with PIH does not appear to be solely related to volume depletion it is probable that other etiologic factors are contributory. For example, Pritchard et al.[2] have suggested that impaired renal function commonly seen in eclamptic women is related to severe regional vasospasm. Our results demonstrating normal pulmonary capillary wedge pressures in oliguric women with PIH are consistent with this hypothesis.

The clinical significance of transient oliguria associated with PIH is controversial. Recently Hankins et al.[3] documented the relationship between elevated pulmonary capillary wedge pressures and delayed spontaneous postpartum diuresis in eclamptic women. They hypothesized that this phenomenon was secondary to the mobilization of extravascular fluid before diuresis. Such observations may account for the occurrence of postpartum cerebral and pulmonary edema in patients with PIH. Under these circumstances oliguria could potentially play an important role in the pathogenesis of these preeclampsia-related complications.

Traditional therapy of PIH-associated oliguria has included the use of diuretics, intravenous fluid challenges, and/or observation. Pritchard et al.[2] have admonished against the use of diuretics or hyperosmolar agents in antepartum women with PIH. They hypothesized that placental blood flow may be compromised by decreasing intravascular volume. Such a further reduction in regional blood flow might adversely affect the fetus, since preeclampsia alone is often associated with uteroplacental insufficiency.

The administration of intravenous fluids in the absence of accurate volume assessment may also be haz-

Table I. Summary of urinary diagnostic indices in oliguria

	Prerenal	*Intrarenal*
Urine osmolality (mosm/kg of water)	>500	<150
Urine sodium (mEq/L)	<20	>40
Urine/plasma urea nitrogen	>8	<3
Urine/plasma creatinine	>40	<20
Renal failure index	<1	>1
Fractional excretion sodium	<1	>1

Adapted from Miller et al.[1]

Table II. Characteristics of seven preeclamptic women studied

Patient No.	*Age (yr)*	*Gestational age (wk)*	*Mean arterial pressure (mm Hg)*	*Proteinuria*
1	22	34	143	3+
2	23	34	125	2+
3	23	32	147	4+
4	25	38	108	3+
5	23	32	127	4+
6	19	29	108	4+
7	15	36	115	2+

Table III. Pulmonary capillary wedge pressures and urinary diagnostic indices from seven preeclamptic women

Patient No.	*Urine output (ml/hr)*	*Pulmonary capillary wedge pressure (mm Hg)*	*Urine osmolality*	*Urine sodium*	*Urine/plasma urea nitrogen*	*Urine/plasma creatinine*	*Renal failure index**	*Fractional excretion of sodium†*
1	26	8	955	119	72	268	0.44	0.3
2	26	14	535	103	38	114	0.90	0.7
3	23	4	627	115	19	108	1.06	0.8
4	23	8	294	86	–	33	2.61	1.8
5	29	10	652	138	26	81	1.70	1.2
6	10	13	608	25	–	315	0.08	0.1
7	25	4	466	99	44	123	0.80	0.6

*Urine sodium divided by urine/plasma creatinine.

†Urine/plasma sodium divided by urine/plasma creatinine times 100.

Table IV. Interpretation of date by urinary diagnostic indices

Patient No.	*Pulmonary capillary wedge pressure (mm Hg)*	*Urine osmolality*	*Urine sodium*	*Urine/plasma urea nitrogen*	*Urine/plasma creatinine*	*Renal failure index**	*Fractional excretion of sodium†*
1	8	PR‡	IR§	PR	PR	PR	PR
2	14	PR	IR	PR	PR	PR	PR
3	4	PR	IR	PR	PR	IR	PR
4	8	IR	IR	–	PR/IR	IR	IR
5	10	PR	IR	PR	PR	IR	IR
6	13	PR	PR/IR	–	PR	PR	PR
7	4	PR/IR	IR	PR	PR	PR	PR

*Urine sodium divided by urine/plasma creatinine.

†Urine sodium divided by urine/plasma creatinine times 100.

‡Oliguria from prerenal causes.

§Oliguria from intrinsic renal damage.

ardous. Our findings suggest that if urine output or urinary diagnostic indices were solely used to guide peripartum fluid management, the majority of these tests would indicate hypovolemia in preeclamptic women. On this basis these patients might inappropriately receive excessive intravenous fluids, placing them at higher risk for pulmonary or cerebral edema.

Invasive hemodynamic monitoring provides valuable information regarding the volume status of preeclamptic women with oliguria. If the pulmonary capillary wedge pressure is normal, the clinician may elect to observe the patient under the assumption that transient renal vasospastic factors are responsible for oliguria. In the presence of prolonged oliguria, the clinician may feel compelled to treat the patient despite a normal range of pulmonary capillary wedge pressures. If regional vasospasm is a major determinant of the PIH-associated oliguria, it might be beneficial to use an agent that could selectively improve renal perfusion. Gerstner and Grunberger[4] studied nine eclamptic patients with oliguria who were treated with intravenous dopamine (3 μg/kg/min) after minimal improvement in urine output despite furosemide or osmodiuretic administration. Although the clinical usefulness of this drug has not been verified, these investigators reported an improvement in urine output following dopamine infusion.

Dopamine is a metabolic precursor of norepinephrine and epinephrine which is produced by the kidney and may act intrarenally as a natriuretic hormone.[5] In low doses, dopamine's β-adrenergic action predominates, leading to increased renal plasma flow and glomerular filtration rate by a specific vasodilatory effect on the kidney vasculature. Huss et al.[6] have demonstrated that the urinary excretion of dopamine is significantly higher in preeclamptic women when compared to normal pregnancies. This observation may be related to the high urine sodium levels uniformly seen in our patients.

The clinician should be cautioned that low urine output alone does not necessarily reflect a hypovolemic state in preeclamptic women. Additionally, the several urinary diagnostic indices cited in this report do not appear to reliably reflect intravascular volume depletion. Finally, from a clinical perspective, the primary treatment of severe PIH and its complications is delivery.

We would like to thank Karen Dorman, R.N., and Marilyn Seibel, R.N., for their meticulous assistance.

REFERENCES

1. Miller TR, Anderson RJ, Linas SL, et al. Urinary diagnostic indices in acute renal failure. Ann Intern Med 1978;89:47.
2. Pritchard JA, Cunningham FG, Pritchard SA. The Parkland Memorial Hospital protocol for treatment of eclampsia: evaluation of 245 cases. AM J OBSTET GYNECOL 1984;148:951.
3. Hankins GDV, Wendel GD, Cunningham FG, Leveno KJ. Longitudinal evaluation of hemodynamic changes in eclampsia. AM J OBSTET GYNECOL 1984;150:506.
4. Gerstner G, Grunberger W. Dopamine treatment for prevention of renal failure in patients with severe preeclampsia. Clin Exp Obstet Gynecol 1980;7:219.
5. Lee MR. Dopamine and the kidney. Clin Sci 1982;62:439.
6. Huss R, Hancock KW, Cope GF, Lee MR: Urine dopamine during normotensive and hypertensive pregnancies and the puerperium. Clin Exp Hypertens 1983;2:317.

Adjunctive magnesium sulfate infusion does not alter metabolic changes associated with ritodrine tocolysis

James E. Ferguson II, M.D., R. Harold Holbrook, Jr., M.D., David K. Stevenson, M.D., Paul A. Hensleigh, M.D., Ph.D., and Daniel Kredentser, M.D.

Stanford and San Jose, California

Magnesium sulfate, an agent whose cellular actions might cause metabolic disturbances, has been used concomitantly with ritodrine hydrochloride for preterm labor tocolysis. Although the profound metabolic effects of β-adrenergic agents have been well described, the possibility that adjunctive magnesium might cause further or unexpected alterations in maternal metabolic parameters has not been fully evaluated. To investigate this question, we prospectively randomized patients, in a blinded fashion, to receive ritodrine plus placebo or ritodrine plus adjunctive magnesium sulfate for preterm labor tocolysis. Serial measurements of potassium, glucose, blood urea nitrogen, and hematocrit were obtained and compared between tocolytic treatment groups. The metabolic changes found were similar in each group and appear to result predominantly from β-adrenergic stimulation with no apparent perturbations caused by the direct cellular actions of magnesium sulfate. From the metabolic standpoint, it appears that the clinician may use adjunctive magnesium sulfate without fear of accentuating or obscuring the expected β-adrenergic–induced alterations in the above-mentioned maternal metabolic parameters. (AM J OBSTET GYNECOL 1987;156:103-7.)

Key words: Magnesium sulfate, ritodrine, preterm labor, tocolysis, metabolic effects

β-Adrenergic agents are widely used in obstetrics in an effort to inhibit preterm labor and thereby prevent premature delivery and the possible accompanying adverse fetal and neonatal sequelae. The maternal metabolic effects associated with the intravenous use of these agents have been documented and include hyperglycemia,[1] hypokalemia,[1] hyperinsulinemia,[1] hyperlactacidemia,[1] and decreased bicarbonate,[2] hematocrit,[2] and colloid osmotic pressure values.[1]

From the Section of Maternal-Fetal Medicine, Departments of Gynecology and Obstetrics and Pediatrics, Stanford University School of Medicine, and the Department of Obstetrics and Gynecology, Santa Clara Valley Medical Center.
This work was supported in part by a Mellon Foundation Fellowship Award.
Received for publication June 13, 1986; revised July 29, 1986; accepted August 14, 1986.
Reprint requests: James E. Ferguson II, M.D., Section of Maternal-Fetal Medicine, Department of Gynecology and Obstetrics, Stanford University School of Medicine, Stanford, CA 94305.

Recently, we[3] and others[4, 5] have investigated the adjunctive use of magnesium sulfate with ritodrine for preterm labor tocolysis. Although some have advocated this combined approach, the possibility of dual-agent tocolysis causing further or unexpected alterations in maternal metabolic parameters has not been fully investigated. In an effort to address this clinically important issue, we prospectively evaluated potassium, glucose, blood urea nitrogen, and hematocrit in a group of patients who were randomly chosen to receive either ritodrine with adjunctive magnesium sulfate or ritodrine plus placebo.

Material and methods

A prospective, randomized, blinded clinical trial was designed in which patients in preterm labor were divided into two treatment groups (Table I). Obstetric management of these patients has been previously described,[3] but salient aspects will be reviewed. Informed

Table I. Characteristics, additional treatments, and associated complications in study patients randomly selected for treatment with ritodrine plus placebo or ritodrine plus magnesium sulfate

	Ritodrine plus placebo	*Ritodrine plus magnesium sulfate*
Maternal age* (yr)	24.8 ± 5.5	24.4 ± 5.0
Parity*	1.0 ± 2.0	0.9 ± 0.9
Gestational age* (wk)	29.6 ± 3.1	30.8 ± 3.0
Cervical dilatation*	1.6 ± 1.1	2.3 ± 1.6
Premature rupture of membranes*	6/17 (35%)	6/23 (26%)
Betamethasone treatment*	12/17 (71%)	14/23 (26%)
Patients with vaginal bleeding	2	2
Medical complications	2	2
Multiple gestation	0	1

*Mean ± SD; differences between groups were not significant by Student's t test or χ^2 analysis, where appropriate.

consent, as approved by the Institution's Research and Human Subjects Review Committee, was obtained from each patient. Patients in preterm labor (regular uterine contractions with a documented change in cervical effacement or dilatation before 36 weeks' gestational age) were randomly assigned to receive either ritodrine with adjunctive magnesium sulfate (N = 28) or ritodrine plus placebo (N = 22) for tocolysis. In addition, the randomization specified the intravenous solution for each patient: 5% dextrose in water, 5% dextrose in half normal saline solution, or lactated Ringer's solution. The infusion containing adjunctive magnesium sulfate consisted of 40 gm of magnesium sulfate in 500 ml of the preselected intravenous fluid, whereas the placebo infusion for "ritodrine plus placebo" patients consisted of 500 ml of the preselected intravenous fluid devoid of magnesium sulfate. Both the magnesium sulfate and placebo solutions were labeled as "study solution" and were visually indistinguishable. Intravenous solutions were prepared by a nurse, not otherwise involved in the patient's care, who selected a sealed envelope from a previously randomized file. Thus the physician and nurse attending the patient were unaware of the contents of the "study solution." The ritodrine solution contained 150 mg of ritodrine hydrochloride in 500 ml of intravenous fluid. The ritodrine solution and "study solution" were infused by separate, continuous infusion devices. The single preselected electrolyte solution was used in each patient and served as the vehicle for the ritodrine solution and the "study solution" as well as for baseline fluid maintenance given to achieve a total infusion of 100 ml/hr.

During treatment, standard practice was followed for the administration of the ritodrine solution.[3] Simultaneously with the initiation of the ritodrine infusion, the study solution infusion was begun at a predetermined rate: 105 ml in the first hour, 60 ml during the second hour, and 30 ml/hr thereafter. Thus, if the study solution contained magnesium sulfate, 8.4 gm of magnesium sulfate was delivered in the first hour, 4.8 gm in the second hour, and 2.4 gm/hr thereafter. Only the ritodrine rate was altered in response to uterine activity; the rate of infusion of the "study solution" (whose contents were unknown to the managing physician and nurse) was identical for all patients.

Laboratory evaluation before tocolysis and at 6, 12, and 24 hours after the initiation of therapy included measurements of hematocrit and serum concentrations of potassium, glucose, and blood urea nitrogen. Maternal and fetal monitoring, use of amniocentesis, and steroid administration as well as the timing or use of oral β-adrenergic agents followed our standard practice and have been previously described.[3, 6]

Hematocrit was measured with a Coulter-S (Coulter Electronics, Hialeah, Florida). Serum potassium and blood urea nitrogen concentrations were determined by an Astra-8 Autoanalyzer (Beckman Instruments, Brea, California). Serum glucose was measured by the glucose oxidase automated method by the Astra-8 Autoanalyzer.

Statistical analysis was performed with the use of χ^2 analysis with Yates' correction for continuity, Student's t test (for paired and unpaired samples as appropriate) or one-way analysis of variance.

Results

Fifty patients were enrolled in the study; 28 were randomized to ritodrine and adjunctive magnesium sulfate and 22 patients received ritodrine plus placebo. Ten patients were withdrawn from the study for the following indications: mature lecithin/sphingomyelin ratio (n = 7), serious tocolytic side effect before 6 hours requiring discontinuation of tocolytic agent (n = 1), chorioamnionitis (n = 1), and gestational diabetes (n = 1). Final analysis of metabolic factors therefore included 40 patients who had completed at least 6 hours of intravenous tocolytic therapy: 23 received ritodrine and adjunctive magnesium sulfate and 17 received ritodrine plus placebo.

Because patients were randomly selected to receive one of three intravenous solutions as well as one of the tocolytic regimens, a comparison was made with the use of one-way analysis of variance of all metabolic values between the different solution groups. Six patients received 5% dextrose in water, 18 patients received 5% dextrose in half normal saline solution, and 16 patients received lactated Ringer's solution. No differences were detected between the three solution groups for any of the values (hematocrit, blood urea nitrogen, glucose,

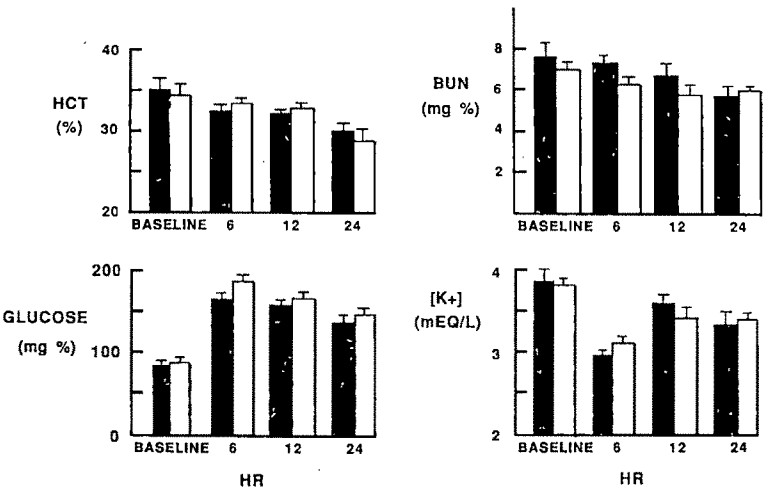


Fig. 1. Comparison of baseline to interval values for hematocrit *(HCT)* and blood urea nitrogen *(BUN)*, glucose, and potassium *([K+])* concentrations. *Dark bars:* Ritodrine plus placebo; *open bars:* ritodrine plus magnesium sulfate. Differences between tocolytic study groups at each time interval were not significant (unpaired *t* test, $p > 0.05$).

Table II. Comparison of maternal baseline values with interval values for hematocrit and blood urea nitrogen, glucose, and potassium concentrations

*Value**	*Baseline (pretreatment)*	*6 hr*	*12 hr*	*24 hr*
Hematocrit (%)	34.7 ± 3.9 (6/18/16‡)	32.7 ± 3.8† (6/18/16)	31.7 ± 3.6† (5/17/12)	29.6 ± 3.7† (5/8/10)
Blood urea nitrogen (mg/100 ml)	7.3 ± 2.1 (6/18/16)	6.6 ± 2.0† (6/18/16)	6.2 ± 1.9† (5/16/11)	5.9 ± 1.2† (5/8/10)
Glucose (mg/100 ml)	87 ± 27 (6/18/16)	175 ± 45† (6/18/16)	159 ± 34† (5/17/12)	133 ± 24† (5/8/10)
Potassium (mEq/L)	3.9 ± 0.4 (6/18/16)	3.1 ± 0.4† (6/18/16)	3.5 ± 0.4† (5/17/12)	3.4 ± 0.4† (5/8/10)

*Values are mean ± SD.
†$p < 0.01$, compared with baseline value (paired *t* test).
‡Number of patients receiving electrolyte solutions A/B/C.

and potassium) at any of the time periods (baseline, 6 hours, 12 hours, and 24 hours). The values for patients in the three solution groups were therefore combined to allow comparison of the two drug regimens.

Comparisons of the four metabolic variables between the two tocolytic treatment groups at each of the four time periods are shown in Fig. 1. No significant differences were noted between the two treatment groups for any of the four variables at any of the time periods ($p > 0.05$, unpaired *t* test).

Since no differences were noted between treatment groups, the groups were then combined to assess changes over a period of time in the metabolic values studied. So that each patient served as her own control, comparisons made included only those patients for whom data were available at both initiation of therapy and the time period being tested. Values for all patients at 6, 12, and 24 hours after initiation of therapy are compared with baseline (pretreatment) values in Table II. Hematocrit, blood urea nitrogen, and potassium values fell and glucose rose significantly at 6 hours after initiation of treatment. Values for all four variables remained significantly different from baseline levels at 12 and 24 hours as well ($p < 0.01$, paired *t* test). To assure that the differences observed over a period of time were not the result of changes in the distribution of patients among the three solution groups, the proportion of patients receiving each solution was compared for 6, 12, and 24 hours; no differences were noted (see Table II).

Comment

The profound metabolic effects of β-adrenergic tocolytic agents are well known. To date, however, the possibility that adjunctive magnesium sulfate might accentuate or obscure metabolic changes has not been

fully considered. Our aim in this report was to determine, by using a prospective, blinded, randomized study, if dual-agent tocolysis consisting of ritodrine and magnesium sulfate might cause further or unexpected changes in maternal metabolic values.

Magnesium sulfate is an effective tocolytic agent with complex cellular mechanisms of action. The major tocolytic action of magnesium sulfate is likely mediated by the extracellular magnesium ion concentration that affects uptake, binding, and distribution of cellular calcium in smooth muscle. It appears that extracellular magnesium ions decrease total exchangeable and membrane-bound calcium at smooth muscle membranes by competing with or displacing calcium ions at cell membrane binding sites.[7] Magnesium sulfate may also have an effect on the cyclic nucleotide system within the cells. Magnesium activates adenylate cyclase, an enzyme involved in the synthesis of cyclic adenosine monophosphate (cAMP).[7] Increases in the levels of cAMP in smooth muscle promote relaxation because (1) cAMP-mediated phosphorylation inhibits the activity of myosin light-chain kinase (the predominant enzyme in the regulation of smooth muscle contractility) and (2) cAMP promotes the accumulation of calcium by the sarcoplasmic reticulum and thus depresses intracellular free calcium concentrations to levels at which the myosin light-chain kinase is not active.[8] The effect of magnesium on cAMP is of particular interest because there are data to suggest that increased cAMP concentrations act to promote vasodilation.[9] If so, then with fluid administration during magnesium tocolysis, one would expect to encounter an expanded intravascular volume. This could be clinically recognized by the finding of a hemodilutional fall in hematocrit.

β-Adrenergic agents also affect fluid balance. The antidiuretic hormone effect of β-adrenergic agents leads to the inability to excrete a sodium load and, coupled with fluid administration, may cause volume overload.[1] Although Hankins et al.,[10] using the pregnant baboon model, could demonstrate no difference in serial hematocrits or colloid oncotic pressures measured in animals receiving intravenous ritodrine, they did note significant increases in sodium and water retention when compared with that in controls. Decreases in hematocrit and colloid osmotic pressure regularly accompany the clinical use of β-adrenergic tocolytic agents in humans.[1,2] Although the potential for an exaggerated effect on hematocrit exists when these agents are used concomitantly, we were unable to demonstrate such an effect (Fig. 1). In our study, the expected decrease in serially monitored hematocrits occurred in both groups. Similarly, blood urea nitrogen values did not differ between groups and decreased in both groups during therapy. Although Philipsen et al.[2] could not demonstrate a significant change in blood urea nitrogen levels during ritodrine treatment, our results are consistent with an expected hemodilutional fall in blood urea nitrogen.

Hyperglycemia was noted in both our patient groups. β-Adrenergic agonists are believed to induce hyperglycemia by increasing the rate of glycogenolysis and/or gluconeogenesis.[1,11] The findings of decreased plasma alanine levels with accompanying increases in glucose levels in patients receiving β-adrenergic therapy support the etiologic role of gluconeogenesis.[12] Magnesium, too, is a controlling and coordinating factor in carbohydrate metabolism. While the administration of physiologic levels of magnesium does not modify blood glucose, high doses of magnesium result in hyperglycemia.[13] This effect is believed to be due to the endogenous release of epinephrine and inhibition of insulin secretion. Additionally, magnesium may affect the affinity and number of insulin receptors. Hatjis et al.[4] recently reported on a group of retrospectively evaluated preterm labor patients in whom magnesium sulfate was added because of ritodrine failure. It is interesting to note in their study that hypokalemia (25 of 30) and hyperglycemia (28 of 30) occurred significantly more often if one combines the two subgroups who received ritodrine and magnesium sulfate and compares them with the patients who received ritodrine alone (19 of 35 and 21 of 35, respectively, $p = 0.0095$ and $p = 0.00156$, Fisher's exact test). Differing results are not unexpected when one considers the dissimilar study designs.

Magnesium concentration is integrally involved in determining the intracellular potassium, calcium, and sodium concentrations.[14] Although the direct effect of magnesium excess on the cell membrane could induce elevated intracellular potassium and resultant hypokalemia, in practice the compensatory mechanisms relating to magnesium and potassium cellular metabolism are so effective that one rarely encounters clinically detectable hypokalemia.[14,15] Richards et al.[11] reported insignificant changes in potassium levels in a small group of women receiving magnesium sulfate for preterm labor tocolysis. Our patients demonstrated similar degrees of hypokalemia in both the adjunctive magnesium and ritodrine plus placebo groups. Cano et al.[16] recently clarified the limited role of β-adrenergic–induced hyperglycemia and resultant hyperinsulinemia in the genesis of hypokalemia. They suggest that hypokalemia is likely a result of stimulation of the β_2-receptors by ritodrine leading to activation of the ionized sodium–ionized potassium adenosine triphosphatase and resultant translocation of potassium across the cellular membrane.[16]

In summary, the adjunctive use of magnesium sulfate, when administered as described herein, does not cause further or unexpected alterations in the maternal

potassium glucose, blood urea nitrogen, or hematocrit values. The metabolic changes found were similar in each group and are predominantly the result of the β-adrenergic stimulation with no apparent perturbations caused by the direct cellular actions of magnesium sulfate. From the metabolic standpoint, it appears that the clinician may use adjunctive magnesium sulfate without fear of accentuating or obscuring the expected β-adrenergic–induced alterations in the above-described maternal metabolic parameters.

We wish to thank Ruby Mei-Lun Wong, Ph.D., for her able assistance with the statistical evaluation of our data.

REFERENCES

1. Benedetti TJ. Maternal complications of parenteral β-sympathomimetic therapy for premature labor. AM J OBSTET GYNECOL 1983;145:1.
2. Philipsen T, Eriksen PS, Lynggard F. Pulmonary edema following ritodrine-saline infusion in premature labor. Obstet Gynecol 1981;58:304.
3. Ferguson JE II, Hensleigh PA, Kredentser D. Adjunctive use of magnesium sulfate with ritodrine for preterm labor tocolysis. AM J OBSTET GYNECOL 1984;148:166.
4. Hatjis CG, Nelson LH, Meis PJ, et al. Addition of magnesium sulfate improves effectiveness of ritodrine in preventing premature delivery. AM J OBSTET GYNECOL 1984;150:142.
5. Ogburn PL, Hansen CA, Williams PP, et al. Magnesium sulfate and beta-mimetic dual-agent tocolysis in preterm labor after single-agent failure. J Reprod Med 1985; 30:583.
6. Ferguson JE II, Hensleigh PA, Gill P. Effects of betamethasone on white blood cells in patients with premature rupture of the membranes and preterm labor. AM J OBSTET GYNECOL 1984;150:439.
7. Altura BM, Altura BT. Magnesium ions and contraction of vascular smooth muscles: relationships to some vascular diseases. Fed Proc 1981;40:2672.
8. Huszar G, Roberts JM. Biochemistry and pharmacology of the myometrium and labor: regulation at the cellular and molecular levels. AM J OBSTET GYNECOL 1982; 142:225.
9. Kramer GL, Hardman JG. Cyclic nucleotides and blood vessel contraction. In: Bohr DF, Somlyo AP, Sparks HV Jr, eds. Handbook of physiology. Vascular smooth muscle. Baltimore: Williams & Wilkins, 1980, vol 2:179-99.
10. Hankins GD, Hauth JC, Kuehl TJ, et al. Ritodrine hydrochloride infusion in pregnant baboons. II. Sodium and water compartment alterations. AM J OBSTET GYNECOL 1983;147:254.
11. Richards SR, Chang FE, Stemple LE. Hyperlactacidemia associated with acute ritodrine infusion. AM J OBSTET GYNECOL 1983;146:1.
12. Lunell NO, Joelsson I, Larsson A, et al. The immediate effect of a beta-adrenergic agonist (salbutamol) on carbohydrate and lipid metabolism during the third trimester of pregnancy. Acta Obstet Gynecol Scand 1977;56:475.
13. Durlach J, Rayssiguier Y. Recent advances on the relationships between magnesium and carbohydrate metabolism. I. Physiological data. Magnesium 1983;2:174.
14. Durlach J, Durlach V. Speculations on hormonal controls of magnesium homeostasis: a hypothesis. Magnesium 1984;3:109.
15. Mordes JP, Wacker WEC. Excess magnesium. Pharmacol Rev 1978;29:273.
16. Cano A, Tovar I, Parrilla JJ, et al. Metabolic disturbances during intravenous use of ritodrine: increased insulin levels and hypokalemia. Obstet Gynecol 1985;65:356.

Radioimmunoscintigraphic detection of occult gestational choriocarcinoma

Richard L. Wahl, M.D., M. B. Khazaeli, Ph.D., Albert F. LoBuglio, M.D., R. A. Patillo, M.D., Michael J. Tuscan, C.N.M.T., and William H. Beierwaltes, M.D.

Ann Arbor, Michigan, Birmingham, Alabama, and Milwaukee, Wisconsin

Localized recurrent gestational choriocarcinoma, evidenced by a rising level of the β-subunit of human chorionic gonadotropin, can be difficult to localize for surgical resection. We describe a patient with a rising level of the β-subunit of human chorionic gonadotropin after extensive therapy for choriocarcinoma in which gamma scanning of the distribution of an intravenously administered iodine 131–labeled monoclonal antibody preparation (5F9.3) allowed localization of a focus of recurrent choriocarcinoma in the lung. Other imaging methods showed this only as a focus of stable scarring. The antibody scan guided surgical resection of this tumor focus, which on gamma counting had a tenfold excess of antibody activity compared with that of the normal lung. This approach may be useful in detecting occult foci of gestational choriocarcinoma for surgical resection in patients with rising levels of the β-subunit of human chorionic gonadotropin and is under further evaluation. (AM J OBSTET GYNECOL 1987;156:108-11.)

Key words: Gestational choriocarcinoma, radioimmunoscintigraphy, monoclonal antibody

Choriocarcinoma, a human chorionic gonadotropin (hCG)–secreting neoplasm of trophoblastic origin (in women), has become increasingly effectively controlled by chemotherapy.[1] While aggressive chemotherapy can frequently cause remissions, even of pulmonary lesions, surgical resection of such lesions may still have a role if such a patient with choriocarcinoma has a persistently elevated level of hCG, has no other metastases, and has contraindications to additional chemotherapy.[1, 2] Detection of such foci of active tumor in the lungs can be difficult, particularly if a patient has pulmonary scarring.[2] While radiolabeled polyclonal anti-hCG antibodies have been used to detect foci of choriocarcinoma in animals and patients, they have not yet been able to detect occult pulmonary disease.[2-5] The use of monoclonal antibodies for radioimmunoscintigraphy may offer advantages over polyclonal reagents.[6-8] We recently applied radioimmunoscintigraphy with an iodine 131–labeled monoclonal antibody originally raised against embryonal testicular cancers (5F9.3), which was unreactive with hCG but reactive with the majority of choriocarcinoma cell lines tested to date, to successfully detect a clinically occult pulmonary focus of choriocarcinoma.[9-11]

From the Division of Nuclear Medicine, Department of Internal Medicine, University of Michigan Medical Center, the Division of Hematology-Oncology, Department of Internal Medicine, University of Alabama in Birmingham, and the Department of Gynecology and Obstetrics, Medical College of Wisconsin.

Supported in part by United States Public Health Service Grant CA33802 awarded by the National Cancer Institute and by D.O.E. Grant No. DE-AC02-76EV02031.

Received for publication February 3, 1986; revised June 30, 1986; accepted August 12, 1986.

Reprint requests: Richard L. Wahl, M.D., Division of Nuclear Medicine, University of Michigan Medical Center, Ann Arbor, MI 48109-0028.

Case report

A 29-year-old woman was referred with a 4-year history of choriocarcinoma. The tumor was first diagnosed in 1981, when she became anemic 2 months after a spontaneous abortion. The anemia was due to uterine rupture caused by choriocarcinoma. Although pelvic disease was largely resected at operation, pulmonary metastases were then noted to be present. Chemotherapy and pelvic irradiation produced a remission by August 1981, but a recurrence was diagnosed 3 months later. Since that time she has had two abdominal operations: (1) removal of the ovaries and (2) resection of a pelvic recurrence. She has also had two right thoracotomies for pulmonary nodule resection and has had a total of 45 cycles of chemotherapy, including bleomycin, methotrexate, actinomycin D, VP-16, and Adriamycin. Pulmonary scarring secondary to the operations and also as a result of the bleomycin therapy has been noted on multiple chest radiographs and computerized tomographic scans. With the chemotherapy, the serum level of β-subunit of hCG (β-hCG) had nearly normalized 4 months before presentation to the University of Michigan Medical Center and chemotherapy was discontinued.

Approximately 2 months before presentation, she was noted to have a rising β-hCG level, which increased further during the next 2 months to 2980 IU when she was admitted to the University of Michigan Medical Center. Three abdominal and chest computerized tomographic scans obtained during the months before admission, including a scan immediately preceding ad-

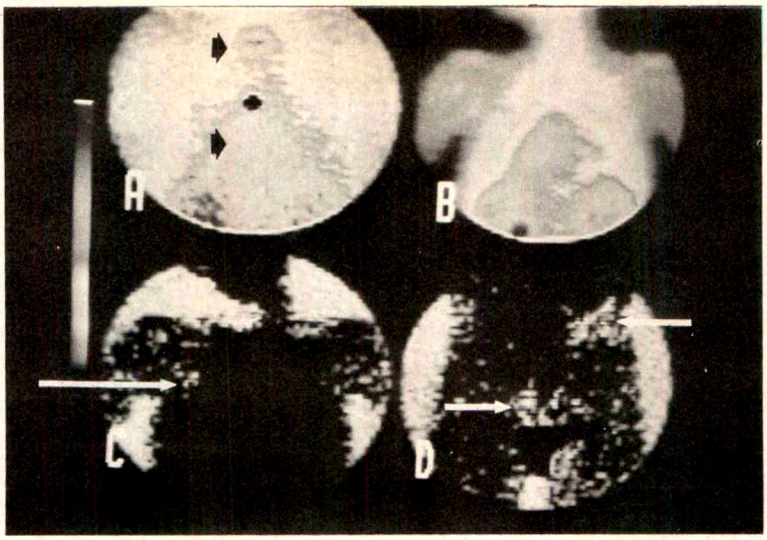


Fig. 1. *A*, ^{131}I-labeled gamma antibody images at 120 hours of posterior chest demonstrating increased activity in thyroid *(upper arrow)*, heart *(lower arrow)*, liver, spleen, and left side of the chest. Black *cross* represents a computer artifact. *B*, Tc99m human serum albumin image obtained 120 hours after antibody infusion and ½ hour after Tc99m human serum albumin injection. This image was obtained simultaneously with *A*, with the use of dual-energy acquisition. *C*, Selected ^{131}I-labeled image with Tc99m human serum albumin background subtracted. Note focal increased activity in left side of the chest *(arrow)*. *D*, ^{131}I-labeled abdominal image (after background subtraction of Tc99m human serum albumin image) showing increased activity in the lower abdomen *(lower arrow)* and in the stomach *(upper arrow)*. (This may be due to free iodine in the stomach and gut as the result of antibody deiodination with subsequent trapping in fluid-filled bowel, caused by partial small bowel obstruction, although the patient had a history of tumor recurring near the bladder.)

mission, were interpreted as "status post hysterectomy with no obvious abdominal disease and stable scarring of the upper lung fields." She was thus admitted to undergo a search for a site of occult disease.

After informed consent was obtained, the patient received 1.25 mCi of ^{131}I-labeled murine monoclonal antibody 5F9.3 intravenously. This represented approximately 1 mg of mouse antibody, labeled by the iodogen technique. The patient was maintained on a regimen of SSKI, three drops/day, throughout the study. She tolerated the injection without adverse effects. Gamma scans with a gamma camera having a large field of view and interfaced to a high-energy collimator were obtained daily until 6 days after injection. These scans, evaluating the entire body of the patient, included 100,000 counts or 20 minutes of acquisition. All data were stored in a dedicated nuclear medicine computer for subsequent analysis. Blood pool subtraction images were generated at 48 and 120 hours after antibody injection, by subtracting the simultaneously acquired Tc99m human serum albumin blood pool image obtained 30 minutes after injection of 1 mCi of Tc99m human serum albumin[5] from the ^{131}I-labeled antibody image. These background subtracted images were then displayed cinematically on a computer monitor with variable levels of background subtraction for final interpretation.[12]

Images examined as above showed a persistent focus of increased activity in the left side of the chest (on posterior images). This was seen best with background subtraction at 48 and 120 hours (Fig. 1, *A* to *C*) and without background subtraction at 6 days after injection. Moderate activity was also noted in the lower abdomen (Fig. 1, *D*), although plain film radiographs obtained at the time of imaging showed a partial small bowel obstruction pattern, with dilated small bowel loops (this problem has been present intermittently since pelvic irradiation).

Review of the chest radiographs in the left upper lung field region, at the site of the increased uptake seen on antibody scan, suggested to some observers the presence of a small nodule in a region of pulmonary scarring; however, the abnormality was extremely subtle and overlay the scapula (Fig. 2). Review of several computerized tomographic scans showed density in the left upper chest corresponding to the area of increased uptake seen on antibody scans. This area had been read longitudinally as representing stable scarring (Fig. 3).

A fine needle aspiration biopsy of the left upper chest scarring was performed 2 weeks after imaging, and the cytologic test results were positive for choriocarcinoma. The slide of this aspirate also underwent gamma counting, and resulting counts were compared with those from a similar-sized aspirate of adjacent normal lung cells from this patient. A tenfold ^{131}I count excess was found in the slide with the aspirate that was positive for tumor when compared with the normal lung aspirate. The patient subsequently underwent a thora-

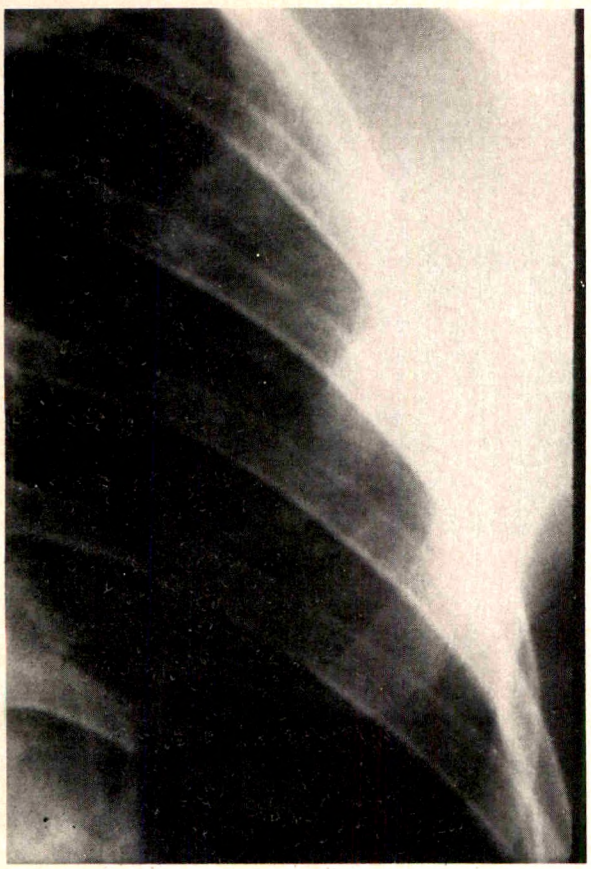


Fig. 2. Close-up view of left upper side of the chest appearing essentially normal in region of increased uptake on antibody scan. On the original film, however, some observers felt a small nodular density might overlie the scapula.

cotomy with resection of tumor. The β-hCG level remained elevated, and she has had two exploratory laparotomies to relieve small bowel obstruction. No abdominal tumor was found at either of these operations. The patient is currently being treated with additional chemotherapy as the β-hCG level remains elevated.

Comment

The ability of radiolabeled antibodies to hCG to localize to choriocarcinoma has been known for some time.[3] More recently, radiolabeled monoclonal and polyclonal antibodies to other tumors have been shown, in certain cases, to be capable of detecting small foci of disease that were undetectable as disease foci by other methodologies. For example, with the use of monoclonal and polyclonal antibodies, occult foci of colon cancer have been detected when computerized tomographic scans were normal.[13, 14] While detection of an occult abdominal focus of choriocarcinoma was reported in 1980 with the use of polyclonal antibodies,[3] this was at a time when computerized tomographic scanning was capable of less spatial and temporal resolution than current devices offer. Detection of "occult" foci of choriocarcinoma has been reported as successful mainly in men, including a series where computerized tomographic scanning was not conducted in all cases.[5] Labeled polyclonal anti-hCG antibodies have been shown to localize to viable choriocarcinoma in women as well, but in all cases other than that reported by Hatch et al.,[4] when there was positive antibody uptake, a definite x-ray or computerized tomographic abnormality highly suggestive of tumor was present.[2, 5]

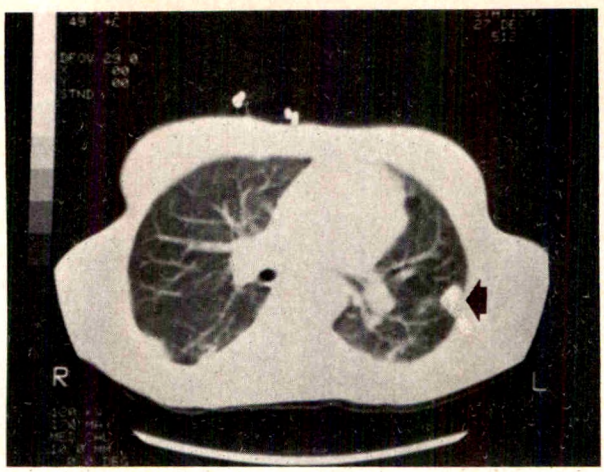


Fig. 3. Computerized tomographic scan of chest obtained 2 weeks before antibody scan demonstrating bilateral upper lobe scarring. Density in left side of the chest *(arrow)* had previously been interpreted as "stable scarring." On needle biopsy and resection this represented a focus of choriocarcinoma and corresponded exactly to the uptake seen on the antibody scan (Fig. 1, *C*).

In the patient described here, radioimmunoscintigraphy was capable of strongly suggesting that a detectable pulmonary abnormality, previously felt to represent scarring, was, in fact, a clinically occult focus of choriocarcinoma. This antibody uptake in the lesion, with biopsy verification, is gratifying and the tenfold excess accumulation over that in the normal lung suggests that it is due to antibody specificity for tumor. Studies in an animal model of choriocarcinoma have shown tumor uptake to be largely due to specific antibody binding.[10] Unfortunately, optimal images could be obtained only after blood pool subtraction, which emphasizes the relatively low tumor/background ratios achieved by many intact monoclonal antibodies.[15] While antibody fragments have helped this somewhat, these low ratios after intravenous antibody delivery represent a problem that must be overcome before more widespread use of this technique as a diagnostic or therapeutic modality can be reasonably considered.[15] Better labeling methods than iodine may also be useful.[16] This point is of particular potential relevance in this case, as increased activity was seen in the lower abdomen when imaging was done in this patient. This imaging oc-

curred at a time of partial small bowel obstruction and it is possible that the increased abdominal uptake was due to deiodination of antibody with accumulation into the bowel. Despite these issues, this considerably encouraging preliminary result, in a case that was difficult to diagnose, adds support to the possibility that radiolabeled monoclonal antibodies will have a role in the diagnosis of malignancies and, in particular, female choriocarcinomas. Studies in additional patients will be essential to define the sensitivity and specificity of this approach.

Thanks are extended to James Botti and Diane Lahti for imaging of patients. Thanks are also due to Steven Kronberg, Linda Laino, Gayle Jackson, Neil Petry, and Jeff Burke for technical assistance and advice in antibody preparation.

REFERENCES

1. Perez CA, Knapp RC, Young RC. Gynecologic tumors. In: DeVita V, ed. Cancer, principles and practice of oncology. Philadelphia: JB Lippincott Company, 1982: 872-4.
2. Begent RHJ, Searle F, Stanway G, Jewkes RF, Jones BE, Vernon P, Bagshawe KD. Radioimmunolocalization of tumours by external scintigraphy after administration of ^{131}I antibody to human chorionic gonadotrophin: preliminary communication. J R Soc Med 1980;73:624-30.
3. Quinones J, Mizejewski G, Beierwaltes WH. Choriocarcinoma scanning using radiolabeled antibody to chorionic gonadotrophin. J Nucl Med 1971;12:69-75.
4. Hatch KD, Mann WJ, Boots LR, Tauxe WN, Shingleton HM, Buchina ES. Localization of choriocarcinoma by ^{131}I-HCG antibody. Gynecol Oncol 1980;10:253-61.
5. Goldenberg DM, Kim EE, DeLand FH, van Nagell JR, Javadpour N. Clinical radioimmunodetection of cancer with radioactive antibodies to human chorionic gonadotropin. Science 1980;208:1284-6.
6. Ballou B, Levine G, Hakala TR, Solter D. Tumor location detected with radioactively labeled monoclonal antibody and external scintigraphy. Science 1979;206:844-6.
7. Berche C, Mach J-P, Lumbroso J-D, et al. Tomoscintigraphy for detecting gastrointestinal and medullary thyroid cancers: first clinical results using radiolabelled monoclonal antibodies against carcinoembryonic antigen. Br Med J 1982;285:1447-51.
8. Wahl RL, Philpott GW, Parker CW. Monoclonal antibody radioimmunodetection of human-derived colon cancer. Invest Radiol 1983;18:58-62.
9. Khazaeli MB, Beierwaltes WH, Pitt GS, Kabza GA, LoBuglio AF. Development and characterization of a monoclonal antibody to human embryonal carcinoma. J Urol (in press).
10. Khazaeli MB. Radioimmunodetection of human embryonic cancer xenografts. Hybridoma 1983;3:89.
11. Pattillo RA, Khazaeli MB, Ruckert ACF, et al. Choriocarcinoma: blocking factor and monoclonal antibody iodine 131 imaging. AM J OBSTET GYNECOL 1984;148:1040-8.
12. Tuscan MJ, Wahl RL, Botti J. Dynamic graded subtraction: a simple method to background correct and display multicompartmental radiopharmaceutical scintigrams. J Nucl Med Technol 1985:23:121-4.
13. Modolfsky PJ, Sears HF, Mulhearn CB Jr, et al. Detection of metastatic tumor in normal-sized retroperitoneal lymph nodes by monoclonal antibody imaging. N Engl J Med 1984;311:106-7.
14. Goldenberg DM, Kim EE, Bennett SJ, Nelson MO, DeLand FH. Carcinoembryonic antigen radioimmunodetection in the evaluation of colorectal cancer and in the detection of occult neoplasms. Gastroenterology 1983;84: 524-32.
15. Wahl RL, Parker CW, Philpott GW. Improved radioimaging and tumor localization with monoclonal F(ab')$_2$. J Nucl Med 1983;24:316-25.
16. Halpern SE, Hagan PL, Garver PR, et al. Stability, characterization, and kinetics of ^{111}In-labeled monoclonal antitumor antibodies in normal animals and nude mouse-human tumor models. Cancer Res 1983;43:5347-55.

Corticosteroid levels in human fetal blood at midgestation and at term

A. López Bernal, E.O.G., D.Phil., and Ian Z. MacKenzie, M.D.
Oxford, England

Cortisol and cortisone were estimated in samples of fetal blood obtained by fetoscopy at 13 to 24 weeks' gestation and in cord blood obtained at elective cesarean section at 38 weeks' gestation. Levels of cortisone exceeded those of cortisol at all gestational ages studied. At midgestation, cortisol levels tended to fall with advancing gestation and had a weak negative correlation with fetal weight. The levels of both steroids were similar in male and female fetuses and increased significantly from midgestation to term. (Am J Obstet Gynecol 1987;156:112-3.)

Key words: Cortisol, cortisone, fetal blood, fetoscopy

Despite the abundant literature on human fetal corticosteroid levels near term, information about corticosteroid levels in fetal blood at midgestation is limited. In a previous study[1] cortisol was measured in samples of cord blood obtained from fetuses delivered by hysterotomy at 15 to 20 weeks' gestation, but there is little information about steroid levels in second-trimester human fetuses before delivery. Pure fetal blood can be obtained at fetoscopy with minimal disturbance to the fetus,[2] and in this article we present data on cortisol and cortisone levels in samples obtained by this technique.

Material and methods

Heparinized cord blood was obtained at fetoscopy in women scheduled for elective abortion. Details of the procedure have been given previously.[2] All women gave informed consent and the study had local ethical approval. Only blood samples from singleton fetuses with no apparent abnormalities were included. Mixed cord blood samples were obtained at elective cesarean sections at term. Cesarean sections were performed because of previous cesarean sections or to deliver infants conceived by in vitro fertilization; all newborn infants were normal.

Plasma samples were separated and stored at −20° C. Cortisol and cortisone were measured by radioimmunoassay after chromatography on Sephadex LH-20.[3] Interassay and intra-assay coefficients of variation were <10% for cortisol and <16% for cortisone.

From the Nuffield Department of Obstetrics and Gynaecology, John Radcliffe Hospital, University of Oxford.
Supported by Action Research, The National Fund for Research into Crippling Diseases, and by Sterling-Winthrop.
Received for publication January 31, 1986; accepted July 28, 1986.
Reprint requests: Dr. A. Lopez Bernal, University of Oxford, Nuffield Department of Obstetrics and Gynaecology, John Radcliffe Hospital, Headington, Oxford, England OX3 9DU.

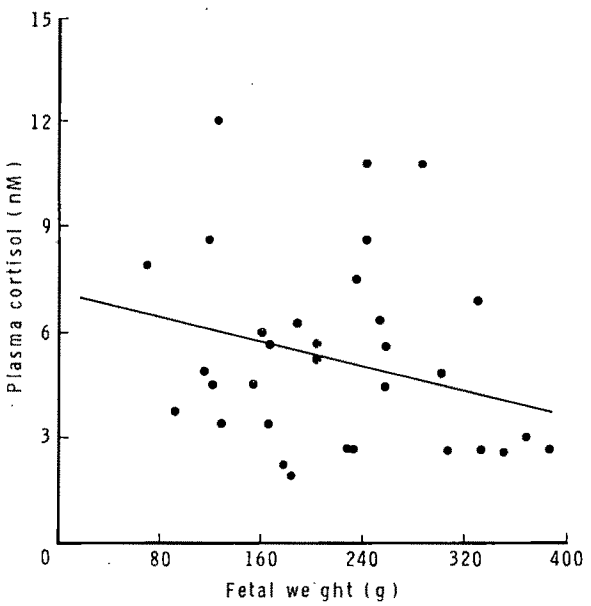


Fig. 1. Correlation between fetal weight (x) and cortisol levels (y) in fetal blood obtained by fetoscopy at 13 to 24 weeks' gestation. Best-fit straight line: $y = 7.01 - 0.01x$; $p > 0.05$.

The data were assessed by the Kruskal-Wallis, Mann-Whitney, and Spearman rank correlation tests.

Results

The data are summarized in Table I. Levels of cortisone exceeded those of cortisol at all gestational ages studied and the levels of both steroids were similar in male and female fetuses. Cortisol and cortisone levels at 38 weeks were significantly higher ($p < 0.001$) than the levels obtained at 13 to 24 weeks' gestation, but the cortisol/cortisone ratio remained similar. At midgestation cortisol levels tended to fall with advancing gestational age and so did the cortisol/cortisone ratio. Overall (Kruskal-Wallis test) these changes were not

Table I. Fetal weight and cortisol and cortisone levels in fetal blood in relation to gestational age

	Gestational age (wk)					
	13-16	*17*	*18*	*19*	*20-24*	*38*
Fetal weight (gm)						
Mean ± SEM	83 ± 16	164 ± 22	201 ± 18	231 ± 23	374 ± 24	3321 ± 186
Range	34-126	95-241	152-302	118-331	305-461	2665-4185
Cortisol (nmol/L)						
Mean ± SEM	7.5 ± 1.1	6.3 ± 1	5.3 ± 0.5	5 ± 0.9	4.6 ± 1.1	50 ± 9
Range	4.4-12.5	1.9-9.1	2.4-7.4	2.6-10.7	2.7-7.6	21-89
Cortisone (nmol/L)						
Mean ± SEM	47 ± 18	17 ± 2	33 ± 16	32 ± 7	42 ± 8	155 ± 33
Range	12-155	9-24	15-63	10-77	21-61	19-367
Ratio cortisol/cortisone						
Mean ± SEM	0.35 ± 0.11	0.44 ± 0.11	0.20 ± 0.04	0.27 ± 0.09	0.12 ± 0.03	0.40 ± 0.06
Range	0.05-0.89	0.16-0.97	0.05-0.33	0.03-1.07	0.04-0.17	0.11-0.69
n	8	8	8	11	4	10

statistically significant. However, in view of previous evidence[1] of a fall in fetal cortisol levels at 18 weeks' gestation, the data were reanalyzed with a comparison of samples obtained before and after 18 weeks, and the differences were significant at $p < 0.05$. Cortisone levels remained relatively stable throughout the midtrimester. The midgestational fall in cortisol levels was also apparent when the results were plotted as a function of fetal weight (Fig. 1) but failed to reach statistical significance (Spearman rank correlation coefficient = -0.26, $p > 0.05$).

Comment

This study provides detailed information on cortisol and cortisone levels in midtrimester fetal blood obtained at fetoscopy. The levels reported here are likely to represent those found in utero in the undisturbed fetus, without the possible stressing effect of delivery. Our data confirm that there is a fall in fetal cortisol levels at about 18 weeks' gestation,[1] but the significance of this fall is weakened by the high p value and by the poor negative correlation between fetal cortisol levels and fetal weight. This apparent fall in fetal cortisol levels has been attributed[1] to the change in the size of the adrenal gland relative to body weight that occurs at midgestation; however, at that stage the bulk of the fetal adrenal cortex composes the "fetal zone," which secretes mainly 3β-hydroxy-5-ene steroids, and cortisol output is relatively small.[4] It is not well established what factors regulate fetal cortisol levels at midgestation, but these levels probably depend on maternofetal exchange as well as fetal adrenal cortisol production. Cortisol levels in maternal blood[5] and in amniotic fluid[6] increase significantly with advancing gestation with no evidence of a fall in the midtrimester.

Comparison of the samples obtained by fetoscopy with those obtained at elective cesarean section at term confirms that there is a true rise in fetal cortisol levels between midgestation and term independent of the influence of labor,[1] and our data show that there is a parallel rise in fetal cortisone levels. In the absence of labor or other factors that might stimulate the fetal adrenal, the cortisol/cortisone ratio in fetal blood would largely be regulated by placental 11β-hydroxysteroid dehydrogenase, and it is of interest that the activity of this enzyme in vitro is similar in midgestation and at term.[7] In summary, our data demonstrate that there is an absolute rise in fetal cortisol and cortisone levels between midgestation and term but the cortisol/cortisone ratio remains stable.

The technical assistance of Mrs. Sue Phipps is gratefully acknowledged.

REFERENCES

1. Murphy BEP. Human serum cortisol levels related to gestational age: evidence of a midgestational fall and a steep late gestational rise independent of sex or mode of delivery. Am J Obstet Gynecol 1982;144:276-81.
2. MacKenzie IZ, Maclean DA. Pure fetal blood from the umbilical cord obtained at fetoscopy: experience with 125 consecutive cases. Am J Obstet Gynecol 1980;138:1214-8.
3. López Bernal A, Anderson ABM, Turnbull AC. Cortisol: cortisone interconversion by human decidua in relation to parturition. Effect of tissue manipulation on 11β-hydroxysteroid dehydrogenase activity. J Endocrinol 1982; 93:142-9.
4. Seron-Ferre M, Lawrence CC, Siiteri PK, Jaffe RB. Steroid production by definitive and fetal zones of the human fetal adrenal gland. J Clin Endocrinol Metab 1978;47:603-9.
5. Rosenthal HE, Slaunwhite WR Jr, Sandberg AA. Transcortin, a corticosteroid binding globulin in plasma. X. Cortisol and progesterone interplay and unbound levels of free steroids in pregnancy. J Clin Endocrinol Metab 1969; 29:352-9.
6. Sippel WG, Mueller-Holve W, Doerr HG, Bidlingmaier F, Knorr D. Concentrations of aldosterone, corticosterone, 11-deoxycorticosterone, progesterone, 17-hydroxyprogesterone, 11-deoxycortisol, cortisol and cortisone determined simultaneously in human amniotic fluid throughout gestation. J Clin Endocrinol Metab 1981;52:385-92.
7. Blasco C, López Bernal A, Turnbull AC. 11β-Hydroxysteroid dehydrogenase activity of the human placenta during pregnancy. Horm Metab Res 1986;18:638-41.

Anticardiolipin antibodies and complement in ninety-nine women with habitual abortion

A. Margareta Unander, M.D., Ph.D., Renée Norberg, M.D., Ph.D., Lennart Hahn, M.D., Ph.D., and Leopold Årfors, M.D.

Göteborg and Stockholm, Sweden

Immunologic investigations were performed on sera from 99 women with habitual abortion (three or more consecutive miscarriages). All were considered healthy and clinical investigations had not revealed any cause for the miscarriages. Sixty-eight were considered to have primary habitual abortion whereas 31 had secondary habitual abortion. Increased anticardiolipin antibody levels were found in 42 patients. None had a primary diagnosis of systemic lupus erythematosus, but at follow-up studies one of them fulfilled the diagnostic criteria of systemic lupus erythematosus. Ten sera, all from patients with primary habitual abortion, showed high anticardiolipin antibody values (≥10 units) concomitant with significantly lower levels of complement factor C4 than those found in sera with moderate or normal anticardiolipin antibody levels. There was no indication of genetic defects explaining the low C4 values, which could be explained at least in part by an activation of complement by the classical pathway. (Am J Obstet Gynecol 1987;156:114-9.)

Key words: Habitual abortion, anticardiolipin antibody, complement factors, antinuclear antibody

Recent studies have revealed a high incidence of repeated fetal loss in patients with systemic lupus erythematosus and concomitant antibodies against negatively charged phospholipids, primarily cardiolipin.[1-6] These antibodies frequently occur in systemic lupus erythematosus and their association with thromboembolic manifestations has been demonstrated.[7] However, high titers of anticardiolipin antibodies have occasionally been found in patients without systemic lupus erythematosus and also in these patients concomitant with thromboembolic episodes. Antibodies to cardiolipin are rarely demonstrated in healthy control subjects but can occasionally be demonstrated, for instance, in patients with acute infections. In these cases, however, the anticardiolipin antibodies seem to occur temporarily whereas in patients with systemic lupus erythematosus the production is constant and apparently rather independent of the disease activity.[8]

We have investigated the occurrence of anticardiolipin antibodies in sera from 99 consecutive female patients referred to us because of recurrent fetal loss. All were considered healthy and no obvious explanation for the abortions had been found. Increased anticardiolipin antibody levels were demonstrated in 42 women, 10 of whom had highly increased values. These 10 patients were followed up with repeated examinations including analyses of the reason for lowered values of complement factor C4 found in the sera.

Material and methods

Patient material. Ninety-nine women who had experienced at least three consecutive abortions, that is, fulfilled the diagnostic criteron of habitual abortion, were sent from all parts of Sweden for immunologic investigations. Clinical investigations including genetic karyotyping of the couples, determinations of antibodies against infectious agents, hysterosalpingography and/or hysteroscopy, and endocrine evaluations, had failed to reveal any cause for the abortions. The women were considered healthy. None of them suffered from any disease that could possibly explain the abortions, such as systemic lupus erythematosus or poorly controlled diabetes. Except for three of them, the women were not pregnant when investigated. Sixty-eight were considered to have primary habitual abortion; they had had three to 11 abortions and no deliveries (Table I). They had experienced a total of 278 pregnancies, six of which (2%) were extrauterine, whereas 221 (79%) had resulted in first-trimester miscarriages and 51 (18%) were second-trimester abortions. The 31 with secondary habitual abortion had been pregnant four to 10 times, and three to nine of the pregnancies had

From the Departments of Obstetrics and Gynecology and Pathology, Sahlgren Hospital, Göteborg, the Department of Immunology, National Bacteriological Laboratory, Stockholm, and the Department of Obstetrics and Gynecology, East Hospital, Göteborg.

Supported by grants from the Medical Research Council of Sweden (Project No. 7326), the Expressen Prenatal Research Foundation, the Göteborg Medical Society, the Swedish Society of Medicine, the Åhlén Foundation, King Gustaf V's 80-year's Research Foundation, and the National Foundation against Rheumatism.

Received for publication May 12, 1986; revised August 12, 1986; accepted August 14, 1986.

Reprint requests: Margareta Unander, M.D., Department of Obstetrics and Gynecology, Sahlgren Hospital, S-413 45 Göteborg, Sweden.

Table I. Pregnancy histories and ages of 99 women with habitual abortion

Type of habitual abortion	*No.*	*Age (yr)*		*No. of miscarriages*	
		Range	*Mean ± SEM*	*Range*	*Mean ± SEM*
Primary	61	20-41	31.0 ± 4.6	3-11	4.7 ± 1.8
Secondary	31	24-42	33.1 ± 4.5	3-10	4.9 ± 1.8

Table II. Distribution of anticardiolipin antibody levels among 99 women with habitual abortion

Anticardiolipin antibody level (units)		*No. of patients*		
IgG	*IgM*	*Primary habitual abortion*	*Secondary habitual abortion*	*Total No.*
0-2	0-2	35	22	57
	3-7	5	2	7
3-4	ND*	10	4	14
5-9	ND*	7	4	11
≥10	ND*	10	0	10
Total No.		67	32	99

*See text for information.

resulted in abortions (see Table I). They had experienced 196 pregnancies, 41 of which (21%) had resulted in deliveries. However, only 30 of 196 pregnancies (15%) resulted in live infants, since 11 of the 41 infants suffered perinatal death due to immaturity, severe growth retardation, or a combination of both. Of the 196 pregnancies, 127 (65%) ended in first-trimester miscarriages whereas 28 (14%) resulted in second-trimester fetal losses. Eight women with secondary habitual abortion had had two deliveries, yet four of them had no living children since they had repeatedly experienced premature deliveries of growth-retarded infants who died during the perinatal period. Two of these eight women had their two infants with previous partners so they indeed had primary habitual abortion in their current relationship. Another woman with secondary habitual abortion had experienced three very premature deliveries of severely growth-retarded infants, only one of whom had survived.

Assay of anticardiolipin antibody. Antibodies against cardiolipin were determined essentially as described by Harris et al.[7] but with the use of an enzyme-linked immunosorbent assay technique and alkaline phosphatase–conjugated antihuman IgG and IgM antibodies, respectively. The results were given in units whereby one unit corresponded to the mean control value plus 1 SD of the mean. Values above or equal to three units were considered pathologic. The control material consisted of healthy blood donors and has been described earlier.[8]

Assay of complement components. Complement components Clq, C3, and C4 were quantified by rocket electrophoresis by means of standard routine techniques.[9] C2 was studied by crossed immunoelectrophoresis as described by Sjöholm and Sturfelt.[10] The presence of C4A and C4B, respectively, was demonstrated by crossed immunoelectrophoresis and by sodium dodecyl sulfate–polyacrylamide gel electrophoresis.[11]

Antinuclear antibodies. Antibodies against nuclear antigens were demonstrated by indirect immunofluorescence with the use of cryostat sections of rat tissue and smears of *Crithidia luciliae* for antibodies to double-stranded deoxyribonucleic acid.

Circulating immune complexes. The Clq and solid-phase conglutinin techniques performed as used by Lambert et al.[12] were used to demonstrate circulating immune complexes in serum.

Statistical calculations. The differences between mean values were analyzed with the use of Student's *t* test for paired comparisons. Significant differences ($p < 0.01$) are indicated in the tables.

Results

Increased anticardiolipin antibody levels were demonstrable in 42 of 99 women with habitual abortion (Table II). Cardiolipin antibodies exclusively of the IgM class were found in sera from seven patients. The IgM values were low (three to seven units). IgG antibodies against cardiolipin were demonstrated in sera from 35 patients, 10 of whom, all with primary habitual abortion, showed values ≥10 units (Table II).

There were no differences in the mean ages (Table I) or numbers of miscarriages (data not shown), respectively, between the various groups of women with different levels of anticardiolipin antibodies. The group of women with high levels of anticardiolipin antibodies (≥10) consisted only of those with primary habitual abortion (Table II). There were no differences

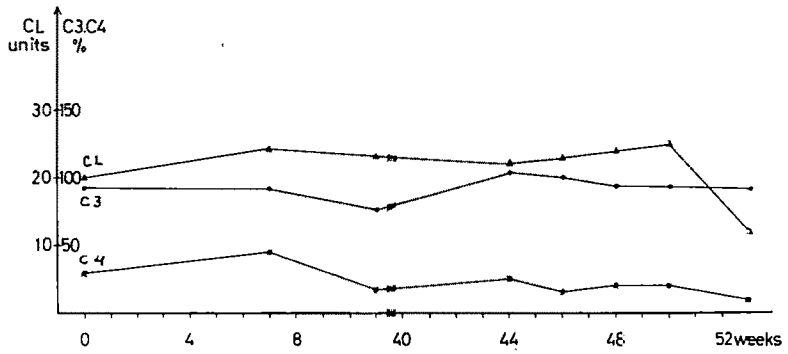


Fig. 1. The levels of anticardiolipin antibodies and complement factors C3 and C4 of Patient P. V., 30 years old. She had suffered miscarriage in pregnancy week 15, 2 weeks before the first blood sample was obtained.

Table III. Mean values of complement factors C3 and C4 in 32 sera containing anticardiolipin antibodies

	Anticardiolipin antibody level		
	3-9 units	*≥10 units*	*Significance*
No.*	22	10	
C3†	106.7 ± 26.7	96.5 ± 22.0	NS
C4†	93.1 ± 31.5	55.7 ± 20.5	$p < 0.01$‡

*Numbers of sera (from different women with habitual abortion).

†Values are given as percent of the normal mean value.

‡Significance level, as tested by Student's *t* test.

in the proportions of primary and secondary habitual abortion between the other groups of women with various levels of anticardiolipin antibodies (Table II).

The levels of complement components C3 and C4 were determined in the first serum specimen available from 32 of 35 patients with IgG anticardiolipin antibodies. As can be seen from Table III the mean value of C4 in sera with anticardiolipin antibody levels of ≥10 units was significantly lower than that in sera with values of anticardiolipin antibody between three and nine units ($p < 0.01$). The mean value of the latter group did not differ significantly from that of healthy control subjects. The mean values of C3 were normal and statistically the same in the two groups.

Antinuclear antibodies were demonstrated in sera from a total of nine patients, four of whom were anticardiolipin antibody negative, one had anticardiolipin antibodies of IgM class only, and the remaining four belonged to the group with IgG anticardiolipin antibody values ≥10 units (Table IV). The antinuclear antibody titers were low (1/10 to 1/25) in sera from all women except one in whom antibodies to double-stranded deoxyribonucleic acid also could be demonstrated. This patient fulfilled the diagnostic criteria of systemic lupus erythematosus although her clinical symptoms were mild and mainly characterized by arthralgia.

The 10 patients with the highest anticardiolipin antibody levels (≥10 units) were followed up with consecutive analyses for at least 12 months. The anticardiolipin antibody levels were rather unaffected by pregnancy and therapy during the observation period as exemplified in Figs. 1 and 2. The normal C3 levels and the low C4 values were also rather constant. During prednisolone treatment in pregnancy some patients displayed a tendency of the C4 values to increase toward normal levels, as illustrated in Fig. 2.

IgG antibodies to cardiolipin were found in subclasses 1 to 4, with certain individual variations of the relative distribution (Table IV).

The cause of the decreased C4 values was studied in sera from the seven patients with the highest anticardiolipin antibody values by analyzing the presence of the two C4 gene products C4A and C4B by crossed immunoelectrophoresis and sodium dodecyl sulfate–polyacrylamide gel electrophoresis. In all serum samples but one the presence of C4A as well as C4B could be demonstrated (Table IV). Thus there was no indication of an increased frequency of two silent C4 alleles within the same locus explaining the low C4 values.

Activation of the early components of the classical pathway was studied in plasma by quantitation of Clq and by measuring the amounts of cleaved C2 by crossed immunoelectrophoresis (Table IV). The Clq values were all within the normal ranges, whereas in all patients investigated cleaved C2, C2a, was demonstrated by crossed immunoelectrophoresis. C2a was not found in normal plasma. Accordingly, the decreased C4 levels could at least partly be explained by an activation of complement by the classical pathway.

Circulating immune complexes were demonstrated by either one or both techniques used in eight of the 10 sera investigated (Table IV).

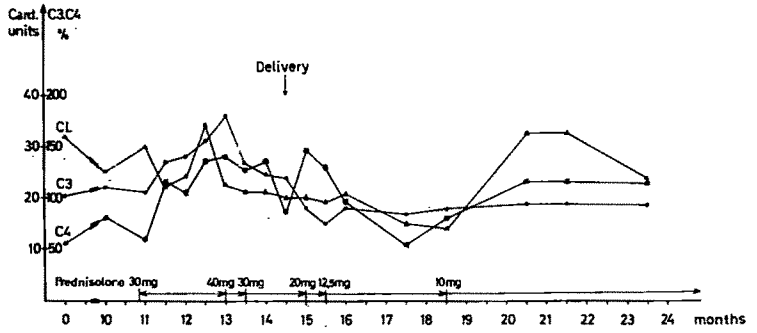


Fig. 2. The levels of anticardiolipin antibodies and complement factors C3 and C4 of Patient E. L., 35 years of age. She had experienced her sixth miscarriage in week 11, 2 months before the first blood sampling. The second blood sample was drawn 10 months later, in pregnancy week 14. Prednisolone treatment was initiated in pregnancy week 17. In week 33 she was delivered by cesarean section of a healthy female infant weighing 1680 gm.

Comment

Our patient material consisted of a consecutively referred nonselected population of habitually aborting women (who had suffered three or more abortions), among whom obvious causes for the repeated abortions had been ruled out by clinical investigations. None of them was considered to have systemic lupus erythematosus when referred to us. It was therefore surprising to find that as many as 42% of them had elevated anticardiolipin antibody values, in 10 cases to very high levels (≥10 units).

Antibodies to cardiolipin were demonstrated in sera from a total of 42 patients. Seven of them had only IgM antibodies and 35 had IgG antibodies. Most of the latter also had demonstrable IgM antibodies. The anticardiolipin activity of purified IgM and IgG fractions was not investigated separately. Thus we do not know to what extent the outcome of the enzyme-linked immunosorbent assay determinations was influenced by the presence of both IgM and IgG antibodies, which might be of different affinity and avidity. Therefore, we have chosen to report the values of IgG anticardiolipin antibody levels. Moreover, in most cases the antibody activity of IgM was lower than that of IgG. The IgG anticardiolipin antibodies were demonstrated within IgG subclasses 1 to 4 (Table IV) although with individual quantitative variations.

Anticardiolipin antibodies are only rarely demonstrated in healthy control subjects.[7,8] Increased levels have been found mainly in patients with systemic lupus erythematosus in whom disease-raised anticardiolipin levels have been associated with thromboembolic manifestations as well as with recurrent abortions. Two of the 99 women with habitual abortion had had deep venous thromboses. They were found in the groups of women with anticardiolipin antibody levels of three to four and ≥10 units, respectively. None of our patients had a primary diagnosis of systemic lupus erythematosus but during the follow-up studies one patient with anticardiolipin antibody levels of ≥10 fulfilled the diagnostic critera of systemic lupus erythematosus.

Moderately increased levels of anticardiolipin antibodies are occasionally found in patients with various infections.[8] In those patients, however, the production of antibodies to cardiolipin seems to be temporary, as investigation of serum samples drawn a year after the original study showed a return to normal levels in all cases but one, in which the patient had a chronic staphylococcal infection.[8] The cause of these elevated anticardiolipin levels is unknown but they may be a part of a polyclonal antibody activation related to the acute infection.

The present anticardiolipin antibody levels were low or moderately elevated in sera from most women with habitual abortion, but in 10 cases (10%), all in women with primary habitual abortion, markedly high values (≥10 units) were registered and seven of the women had anticardiolipin values between 20 and 40 units. According to our experience levels of ≥10 units are very rare in patients with acute infections and values of ≥20 units are only occasionally seen in randomly selected patients with systemic lupus erythematosus and, if so, symptoms compatible with those of thromboses are present.[8] Our patients were followed up with repeated analyses during 15 to 48 months. As seen in Figs. 1 and 2 the anticardiolipin antibody levels were rather constant and independent of pregnancy and steroid therapy during the study. The same observation has been made in patients with systemic lupus erythematosus in whom the anticardiolipin antibody values usually are unrelated to disease activity and therapy.[8]

There were no differences in the mean ages (Table I) or numbers of miscarriages, respectively, between the various groups of women with different levels of

Table IV. Anticardiolipin antibodies and complement components

Patients	IgG anticardiolipin antibodies (units)	Presence of anticardiolipin antibodies				C3 (%)
		IgG subclass 1	IgG subclass 2	IgG subclass 3	IgG subclass 4	
L.S.	17	+	−	+	+	64
E.L.	32	+++	++	+	++	87
M.P.	21	+++	++	++	+	94
A.-K.N.	29	+++	+++	−	+	93
M.B.	28	+++	(+)	−	+	92
A.E.	37	+	+++	−	+	86
P.V.	24	+	+++	−	++	105
U.G.	17	+++	++	++	+	112
A.N.	10	++	+	−	+	140
S.O.	12	++	−	+	+	112

anticardiolipin antibodies. The group of women with high anticardiolipin antibody levels (≥10) consisted only of those with primary habitual abortion (Table II). The proportions of primary to secondary habitual abortion were the same, respectively, in the other groups of women with various levels of anticardiolipin antibodies (Table II).

Typical of most sera with highly raised anticardiolipin values were levels of complement factor C4 that were below or at the lower normal range whereas most of the C3 levels were well within the normal ranges (Table III). With few exceptions the same observations have also been made in patients with thromboembolic manifestations and high anticardiolipin levels (Norberg R, et al., unpublished observations).

The cause of the C4 decrease is not entirely clear. The production of C4 is governed by two gene loci, C4A and C4B, on chromosome six. About 20% of patients with systemic lupus erythematosus have been reported to have two silent alleles within the same locus in comparison with <5% in healthy control subjects.[13] Since only one of the 10 patients with high levels of anticardiolipin antibody lacked one of the gene products there was no obvious evidence for an increased incidence of genetic defects explaining the low C4 levels in these 10 patients. On the other hand, the C2 cleavage demonstrated by crossed immunoelectrophoresis indicated an increased complement activation by the classical pathway. The C3 values were with some exceptions normal, but this does not, however, exclude that the turnover of C3 also was increased. To what extent reactions between anticardiolipin antibodies and corresponding antigens contributed to the complement activation has not been investigated. Most of these 10 sera contained circulating immune complexes but the structure of these complexes has not been studied.

It was, however, remarkable that the C4 values remained low during the observation period in most cases. The patients had no clinical symptoms and they considered themselves healthy. The same observation has also been made in other patients, who have been followed up for several years after an acute thromboembolic episode.[8] They had continuously high levels of cardiolipin antibodies and low C4 values, which remained so during a long observation period without any signs of new thromboses[8] (Norberg R, et al., unpublished data).

The persistingly very high anticardiolipin antibody levels and signs of complement activation via the classical pathway as well as the increased frequency of antinuclear antibodies in 10 of the women with primary habitual abortion indicate that these women compose a special group exhibiting immunologic reactions with features similar to those of systemic lupus erythematosus. This justifies that these patients will be carefully surveyed with regard to the development of clinical symptoms of systemic lupus erythematosus.

The women with low or moderately increased levels of anticardiolipin antibodies did not stand out clinically from those with normal levels. There is no apparent explanation of their elevated anticardiolipin antibody levels. The women were not pregnant at the time of investigation and the time since the last miscarriage varied. The influence of pregnancy and abortion on the anticardiolipin antibody levels cannot be ruled out, the more so as the incidence of increased values during normal pregnancy is not known.

Negatively charged phospholipids as cardiolipin are constituents of all cell membranes. Antibodies to cardiolipin have been attributed pathogenic importance by interacting with, for example, platelets and vascular endothelial cells. In this way mechanisms may be provoked that can lead to an increased tendency for platelet aggregation and other cellular events of importance for the coagulation process.[3, 6] There is, however, no definite support for the direct pathogenic role of antiphospholipid/anticardiolipin antibodies either in patients with systemic lupus erythematosus[8] or in the pres-

C4 (%)	*Presence of C4A*	*Presence of C4B*	*Clq (%)*	*Presence of C2a*	*Presence of circulating immunoglobulin complexes*
54	+	+	103	++	+
57	+	+	94	++	+
63	+	+	112	++	+
43	+	−	122	ND	+
78	+	+	91	(+)	−
36	+	+	99	++	+
25	+	+	112	+++	+
58	+	+	94	++	−
79	+	+	113	+	+
67	+	+	98	+	+

ent group of aborting women. Treatment with prednisolone and minute doses of acetylsalicylic acid has resulted in living infants[1, 4] (Fig. 2), but the anticardiolipin levels were remarkably uninfluenced by the medication (Fig. 2) whereas the values of activated partial thromboplastin time decreased.[1, 14]

Eight of these women with habitual abortion with elevated anticardiolipin antibody levels (two of them had ≥10 units) who had no demonstrable blocking antibody received leukocyte transfusions[15] and subsequently had successful pregnancies without any therapy aimed at diminishing the autoimmune aberration. Therefore, according to our experience, even the occurrence of high levels (≥10 units) of anticardiolipin/antiphospholipid antibody in a woman with habitual abortion is not a definite reason for treatment with prednisolone and low-dose acetylsalicylic acid during pregnancy, which has been suggested.[1] Treatment should be considered in those women in whom the sera during a long period of time constantly display very elevated anticardiolipin levels preferentially concomitant with low C4 values, prolongation of activated partial thromboplastin time, and strong blocking antibody[15] (Unander AM, et al., unpublished data).

The skillful technical assistance of Mrs. Katarina Krook and Mrs. Katarina Karlén is gratefully acknowledged.

REFERENCES

1. Lubbe WF, Butler WS, Palmer SJ, Liggins GC. Fetal survival after prednisolone suppression of maternal lupus anticoagulant. Lancet 1983;1:1361-3.
2. Derue GJ, Englert HJ, Harris EN, et al. Fetal loss in systemic lupus: association with anticardiolipin antibodies. J Obstet Gynaecol 1985;5:207-9.
3. Hughes GRV. Thrombosis, abortion, cerebral disease, and the lupus anticoagulant. Br Med J 1983;287:1088-9.
4. Lockshin MD, Druzin ML, Goei S, et al. Antibody to cardiolipin as a predictor of fetal distress or death in pregnant patients with systemic lupus erythematosus. N Engl J Med 1985;313:152-6.
5. Branch WB, Scott JR, Kochenour NK, Hershgold E. Obstetric complications associated with the lupus anticoagulant. N Engl J Med 1985;313:1322-6.
6. de Wolf F, Carreras LO, Moerman P, Vermylen J, van Assche A, Renaer M. Decidual vasculopathy and extensive placental infarction in a patient with repeated thromboembolic accident, recurrent fetal loss, and a lupus anticoagulant. Am J Obstet Gynecol 1982;142:829-34.
7. Harris EN, Gharavi AE, Boey ML, et al. Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. Lancet 1983;2:1211-4.
8. Sturfelt G, Nived O, Norberg R, Torstensson R, Krook K. Antibodies to cardiolipin in SLE. Arthritis Rheum 1986 (in press).
9. Sjöholm AG. Complement components in normal serum and plasma quantitated by electro-immunoassay. Scand J Immunol 1975;4:25-30.
10. Sjöholm AG, Sturfelt G. Cleavage of C2 in pathological serum and plasma studied by crossed immunoelectrophoresis. Acta Pathol Microbiol Immunol Scand [C] 1984;92:265-9.
11. Roos MH, Mollenhauer E, Démant P, Rittner C. A molecular basis for the two locus model of human complement component C4. Nature 1982;298:654-6.
12. Lambert PH, Dixon FJ, Zabler RH, et al. A WHO collaborative study for the evaluation of eighteen methods for detecting immune complexes in serum. J Clin Immunol 1978;1:1-15.
13. Dawkins RL, Christiansen FT, Kay PH, et al. Disease association with complotypes, supratypes, and haplotypes. Immunol Rev 1983;70:5-22.
14. Lockshin MD, Druzin ML. Letter. N Engl J Med 1985; 313:1351.
15. Unander AM, Lindholm A. Transfusions of leukocyte-rich erythrocytes: a successful treatment in selected cases of habitual abortion. Am J Obstet Gynecol 1986; 154:516-23.

Fetal blood group determination in first-trimester pregnancy for the management of severe immunization

Humphrey H. Kanhai, M.D., Jack Bennebroek Gravenhorst, M.D., Reinoud J. Gemke, M.D., Marijke A. Overbeeke, M.S., Luigi F. Bernini, M.D., and Geoffrey C. Beverstock, M.D.

Leiden and Amsterdam, The Netherlands

The presence or absence of Rh_o (D) and Kell antigens on fetal red blood cells was determined in the first trimester of pregnancy on erythrocytes obtained by chorionic villi sampling with the use of mixed agglutination and solid phase microimmunofluorescence techniques. Pregnancies in one Kell-sensitized woman and seven severely RH-sensitized women with a poor obstetric history and a partner heterozygous for the offending antigen were examined. A conclusive diagnosis could be made in seven of the eight cases studied. (AM J OBSTET GYNECOL 1987;156:120-3.)

Key words: Rh immunization, Kell sensitization, chorionic villus sampling, first-trimester fetal blood group typing, mixed agglutination; microimmunofluorescence

The technique of chorionic villus sampling was introduced in the 1970s[1] and has become widely used for the diagnosis of genetic disorders in the first trimester of pregnancy. Apart from syncytiotrophoblast, cytotrophoblast, and connective tissue, the villous material obtained by chorionic villus sampling between the eighth and eleventh weeks of gestation contains distinct fetal vessels.[2] This offers the opportunity to study blood group antigens involved in hemolytic disease of the fetus and the newborn infant.

Erythroblastosis fetalis resulting from incompatibility of the Rh_o (D) antigen still occurs despite the introduction and wide application of anti-Rh_o (D) immune globin prophylaxis.[3] In addition, severe hemolytic disease of the newborn infant can be caused by other, so-called irregular blood group incompatibilities. To prevent adverse perinatal outcome, severely immunized patients are managed with a variety of invasive diagnostic and therapeutic procedures. In the event that the partner of such a patient is heterozygous for the blood group antigen involved, knowledge of the fetal blood group in early pregnancy permits a more rational management of the index pregnancy. If the fetus lacks the offending paternal antigen, unnecessary diagnostic and therapeutic procedures can be prevented. On the other hand, if the fetus appears to be positive, the choice can be either to terminate pregnancy or to initiate specific diagnostic and therapeutic procedures early in pregnancy. This report is part of an ongoing study investigating the expression of various blood group antigens in chorionic villus material in the first trimester of pregnancy; methodologic aspects of it have been reported on previously.[4, 5] The present report deals with the chorionic villus sampling technique used, the preparation of a cell suspension containing fetal erythrocytes, and the techniques for antigen detection in eight severely sensitized pregnancies.

From the Departments of Obstetrics and Gynecology and Human Genetics, University Hospital Leiden, and the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, incorporating the Laboratory for Experimental and Clinical Immunology of the University of Amsterdam, The Netherlands.

Received for publication May 30, 1986; revised July 29, 1986; accepted August 14, 1986.

Reprint requests: Dr. H. H. H. Kanhai, Department of Obstetrics and Gynecology, University Hospital Leiden, Rijnsburgerweg 10, 2333 AA Leiden, The Netherlands

Patients and methods

Patients. One woman severely immunized against the K antigen and seven women severely immunized against the Rh_o (D) antigen with a poor obstetric history and a heterozygous partner requested first-trimester fetal blood group determination. Six of them had requested termination of pregnancy unless the fetus could be shown to be negative for the blood group antigen in question. The characteristics of the eight patients are listed in Table I. Four of them had experienced at least one perinatal loss due to the blood group antagonism. The other four women had been delivered of at least one infant with severe erythroblastosis fetalis.

Chorionic villus sampling. Between the eighth and eleventh weeks of gestation, a transcervical trophoblast biopsy was taken with a flexible cannula with a diameter of 1.5 mm (Portex or KCH cannula). Under real-time ultrasound guidance, the tip of the cannula was directed to the insertion of the umbilical cord in the chorion frondosum. The obturator was withdrawn, a 20 ml syringe was attached to the catheter, and about 15

Table I. Characteristics of patients

	Patient No.							
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Parity	2	3	2	2	1	3	3	1
Immunization	Rh_o (D/C)	Rh_o (D)	Rh_o (D)	K	Rh_o (D/E)	Rh_o (D)	Rh_o (D/C)	Rh_o (D)
Indirect antiglobulin test	4000/32	2000	8000	1000	128/4	4000	4000/32	512
ADCC assay* (%)	>100	>100	>100	82	78	>100	>100	87
Partner's phenotype	CcDee	CcDee	CcDee	Kk	ccDEe	ccDEe	CcDee	CcDee

*ADCC: Antibody-dependent cell-mediated cytotoxity.[16]

Table II. First-trimester fetal blood group determination in eight patients with severe immunization

Patient No.	*Indication for chorionic villus sampling*	*Weeks' gestation at chorionic villus sampling*	*Technique**	*Fetal blood group factor*	*Results of follow-up*	*Confirmation of biopsy diagnosis*
1	Rh_o (D)	9	I	Rh_o (D/C) positive	Termination at 10 wk	Yes
2	Rh_o (D)	8-10	I	Rh_o (D) positive	Termination at 11 wk	Yes
3	Rh_o (D)	9-11	I	Rh_o (D) negative	Delivery at term	Yes
4	Kell	9	I	K positive	Delivery at 31 wk	Yes
5	Rh_o (D)	10	I + II	Rh_o (D/E) negative	Delivery at term	Yes
6	Rh_o (D)	9-11	I + II	Rh_o (D) negative (?)†	Delivery at term	Rh_o (D) negative
7	Rh_o (D)	10	I + II	Rh_o (D/C) positive	Termination at 11 wk	Yes
8	Rh_o (D)	10	I + II	Rh_o (D) positive	Delivery at 35 wk	Yes

*I: Mixed agglutination technique; II: solid phase microfluorescence technique.
†No conclusive diagnosis possbile because the majority of fetal erythrocytes were damaged.

ml of suction was applied. The biopsy specimen was flushed out into a Petri dish containing heparinized saline solution. The material was immediately examined under a dissecting microscope and considered to be adequate when 20 mg (or more) of vascularized chorionic villi was obtained.

Isolation of fetal erythrocytes. Maternal tissue (mucus and decidua) was removed with a Pasteur pipette leaving the aspirate containing fluid (portion 1) and trophoblast. The trophoblast was isolated, minced, and washed in heparinized saline solution (portion 2). The minced villi were collected for culture overnight in Ham's F10 medium. After 24 hours the culture fluid was removed and examined (portion 3).

About 1 ml of each of the portions 1, 2, and 3 was stained with a fluorescent antifetal hemoglobin antiserum.[6] With phase contrast and fluorescence microscopy, fetal erythrocytes can be differentiated from maternal cells because of the intensity of their fluorescence and their larger size, thus allowing the percentage of fetal erythrocytes in the biopsy specimen to be calculated.

Determination of fetal blood group antigen. The presence of Rh_o (D)–positive erythrocytes in the biopsy material can be demonstrated by a mixed agglutination technique for the detection of a minor erythrocyte population.[7] A modification of this technique was used when dealing with Rh antigens[8]; for Kell determination we used the original technique.[7] In the presence of Rh_o (D)–positive erythrocytes, rosette formation occurs after centrifugation. The sensitivity of this technique in our application is at the level of one positive fetal erythrocyte per 1024 maternal Rh_o (D)–negative cells.[5]

At first, the mixed agglutination technique was used, but to overcome limitations of sensitivity and specificity of the mixed agglutination test, we developed a solid phase microfluorescence technique to detect the minor populations of positive erythrocytes. This technique is described in detail elsewhere[5] and allows ABO and Rh phenotypes of a minor cell population to be determined at the ratio of 1 in 4000. This microfluorescence technique was used in the last four cases of this study.

Results

Results of the eight pregnancies studied are summarized in Table II. In Case 1, 40 mg of trophoblast tissue was obtained after one sampling attempt. All three portions contained fetal red blood cells and the mixed agglutination technique showed two rosettes per 100 total red blood cells, indicating that the fetus was Rh_o (D) positive. One week after chorionic villus sampling the pregnancy was terminated by suction curettage and the presence of an Rh_o (D)–positive fetus was confirmed by both standard and mixed agglutination techniques.

In Case 2, the first chorionic villus sampling was attempted at 8 weeks of gestation. A total of 40 mg of villous tissue was obtained. Although the villi contained

several blood vessels, fewer than one fetal erythrocyte per 1000 maternal red blood cells was present in portions 1 to 3, and typing of the fetal cells was impossible. Clotting in the villous vessels was a possible cause of this failure. The chorionic villus sampling was repeated 10 days later when portion 3 contained 2% fetal erythrocytes with rosette formation in the mixed agglutination technique. This pregnancy was terminated at 11 weeks and the diagnosis was confirmed.

In Case 3, only the second chorionic villus sampling attempt produced sufficient chorionic villi but an insufficient number of fetal erythrocytes. The third attempt at 11 weeks afforded percentages of fetal erythrocytes in portions 1, 2, and 3 of 0.2%, 1.5%, and 0.2%, respectively. No rosette formation could be detected in the mixed agglutination technique, indicating an Rh_o (D)–negative fetus. After an uneventful pregnancy and spontaneous labor at term a healthy Rh_o (D)–negative female infant (3780 gm) was born.

Case 4 is the first reported case of typing for K in the first trimester of pregnancy. The first biopsy provided only 10 mg of chorionic villi and a second catheter insertion was undertaken to ensure that enough fetal erythrocytes were collected for the mixed agglutination technique. Our study had thus far shown that the K antigen cannot be detected by the microfluorescence technique.[5] This second biopsy attempt resulted in a decidual hematoma and the procedure was immediately terminated. Rosettes (0.5% to 1%) were seen in the preparations and a likely diagnosis of a K-positive fetus was made. The patient decided to continue her pregnancy and weekly plasmaphereses were started. The pregnancy was complicated by recurrent vaginal bleeding and preterm rupture of the membranes after the first intrauterine transfusion at 23 weeks. In total, four intrauterine transfusions were necessary. At 31 weeks of gestation a healthy female infant weighing 1560 gm was born by elective cesarean section. The neonatal period was uneventful apart from the need for several blood transfusions. In Case 5, 60 mg of chorionic villi was obtained after one sampling attempt. In portion 2, which contained 2% fetal erythrocytes, no rosettes were detected in the mixed agglutination test. Microfluorescence revealed no reaction with anti–Rh_o (D) or anti–Rh_o (E), indicating that the fetus was Rh_o (D) and Rh_o (E) negative. The patient was referred to her obstetrician at a district hospital near her home. After an uneventful pregnancy and induction of labor at 39 weeks of gestation, a healthy Rh_o (D)– and Rh_o (E)–negative male infant weighing 3460 gm was born.

In Case 6, chorionic villus sampling was technically difficult and resulted in 5 mg of villi after four attempts. Although the percentage of fetal erythrocytes was 0.8%, a conclusive diagnosis was impossible (Table II). The patient refused further chorionic villus sampling attempts. Her pregnancy was uneventful. At term she was delivered of a healthy Rh_o (D)–negative male infant weighing 3525 gm.

In Case 7, two biopsy attempts provided 25 mg of villous material. Both the mixed agglutination and the immunofluorescence tests were positive for anti-Rh_o (D) and the microfluorescence technique also was positive for anti–Rh_o (C). This patient decided to terminate the pregnancy and the results were confirmed in the abortion material.

In Case 8, one biopsy attempt produced 60 mg of tissue with 1% fetal erythrocytes in portions 1, 2, and 3. The results of both the mixed agglutination and the microfluorescence techniques indicated that the fetus was Rh_o (D) positive. This patient decided to continue her pregnancy and weekly plasmaphereses were started. After three intrauterine transfusions labor was induced at 35 weeks of gestation. A healthy female infant weighing 2700 gm was born and subsequently did well.

Comment

The results of our study thus far have demonstrated the feasibility of Rh and Kell typing of fetal erythrocytes obtained by chorionic villus sampling. With a modified mixed agglutination technique[7,8] and microfluorescence,[5] a conclusion about the fetal blood group was possible in seven of the eight cases studied. In one patient the Rh_o (D) status of the fetus could not be determined, although there were 0.8% fetal erythrocytes in the chorionic villus sampling material. Chorionic villus sampling was difficult in this obese patient; although no rosettes were seen and fluorescence was negative, a conclusive diagnosis was impossible because of damaged fetal erythrocytes. However, pregnancy was uneventful and the child was Rh_o (D) negative. In the two cases in which the fetus was Rh_o (D) negative, pregnancies followed a natural course. Of the five pregnancies in which the fetus was Rh_o (D) positive, three were terminated with suction curettage with the patient under local anesthesia. In the remaining two pregnancies, plasmapheresis and intensive management were started at 10 to 11 weeks.

The laboratory techniques (mixed agglutination and microfluorescence) are based on the principle of the detection of a blood group on a minor (fetal) red blood cell population that is not present on the major (maternal) red blood cell population. As the majority of erythrocytes in all biopsy specimens are of maternal origin, only the demonstration of rosettes or fluorescence is conclusive. In cases in which there is no rosette formation and the fluorescence is negative, on the other hand, it can only be concluded that the fetus is negative for the blood group under investigation if there are an

adequate number of fetal erythrocytes in the biopsy specimen. A further chorionic villus sampling attempt can be necessary in such cases.

Determination of a fetal blood group in the first trimester of pregnancy is of great advantage for severely immunized patients with a heterozygous partner. Until recently, the antenatal determination of fetal blood group was only possible from the second trimester on by fetoscopy[9] and ultrasound-guided percutaneous needle puncture of an umbilical vessel.[10] The risks of fetal blood group determination in the first trimester by chorionic villus sampling are probably less than in second-trimester procedures. Moreover, chorionic villus sampling has the advantage that an early diagnosis is possible. However, the risks of chorionic villus sampling must also be considered. In 11,819 pregnancies (90 centers) the maximal fetal loss rate after chorionic villus sampling was 4%.[11] Since the obstetric risks of chorionic villus sampling are still not fully understood, the technique must presently be considered as an applied research procedure,[12, 13] which requires further evaluation. In our group of eight patients, one pregnancy was probably complicated as a result of the procedure. Another risk of chorionic villus sampling for this type of patient is fetomaternal transfusion resulting in an increase of antibody levels. In our group of eight patients there were two with sensitized fetuses who wished to continue their pregnancies. No significant rise in antibody levels was detected in these patients. Although chorionic villus sampling is known to cause a rise of α-fetoprotein in a significant number of patients, this is by no means conclusive evidence of fetomaternal hemorrhage.[14]

Termination of pregnancy after chorionic villus sampling when the fetus is found to be Rh_o (D) positive deserves special comment. Since anti-Rh_o (D) serum does not exist, the likelihood that a subject is heterozygous for Rh_o (D) can be estimated only after the rhesus phenotype has been determined.[15] This implies a chance of error in the estimation of heterozygosity for Rh_o (D). Other diagnostic tools, such as the obstetric history and phenotyping of sibs of the partner, are important for a more definitive conclusion concerning the paternal genotype.

When all these considerations are taken into account, chorionic villus sampling for fetal blood group typing must be reserved for a highly selected group of patients, who will require detailed follow-up. Good counseling before pregnancy remains of paramount importance.

Our thanks are due to Mr. R. J. Meerman for expert ultrasonic assistance and to Prof. C. P. Engelfriet and Prof. M. J. N. C. Keirse for their support.

REFERENCES

1. Department of Obstetrics and Gynaecology, Tietung Hospital of Anshaw Iron and Steel Company. Fetal sex prediction by sex chromatin of chorionic villi cells during early pregnancy. Chin Med J 1975;1:117.
2. Arey LB. Developmental anatomy. A textbook and laboratory manual of embryology. 8th ed. Philadelphia: WB Saunders, 1974.
3. Bennebroek Gravenhorst J, Woodfield DG. Isoimmunisation in pregnancy: diagnosis, treatment and prevention. In: Das PC, Smit Sibenga Cth, Halie MR, eds. Supportive therapy in haematology. The Hague: Martinus Nijhoff, 1985:129.
4. van t Veer MB, Overbeeke MAM, Geertzen HGM, van der Lans SMGA. The expression of Rh-D factor in human trophoblast. AM J OBSTET GYNECOL 1984;150:1008.
5. Gemke RJBJ, Kanhai HHH, Overbeeke MAM, et al. ABO and rhesus phenotyping of fetal erythrocytes in first trimester of pregnancy. Br J Haematol (in press).
6. Wood WG, Stamatoyannopoulos G, Lim G, Nute PE. F-cells in the adult: normal values and levels in individuals with hereditary and acquired elevation of HbF. Blood 1975;46:671.
7. Jones AR, Silver S. The detection of minor erythrocyte populations by mixed agglutinates. Blood 1958;13:763.
8. Sebring ES, Polesky HF. Detection of fetal maternal hemorrhage in Rh immunoglobulin candidates. Transfusion 1982;22:468.
9. MacKenzie I, Guest CM, Bowell PJ. Fetal blood group studies during mid trimester pregnancy and the management of severe Rhesus isoimmunization. Prenat Diagn 1983;3:41.
10. Daffos F, Capella-Pavlovsky M, Forestier F. Fetal blood sampling during pregnancy with use of a needle guided by ultrasound: a study of 606 consecutive cases. AM J OBSTET GYNECOL 1985;153:655.
11. Jackson LG. CVS latest news. Thomas Jefferson University Bulletin 1986 March 6.
12. Modell B. Chorionic villus sampling. Evaluation, safety and efficacy. Lancet 1985;1:737.
13. Keirse MJNC, Kanhai HHH, Bennebroek Gravenhorst J. Safety of chorionic villus sampling. Lancet 1985;2:1312.
14. Warren RC, Butler J, Morsman JM, McKenzie C, Rodeck CH. Does chorionic villus sampling cause fetomaternal haemorrhage? Lancet 1985;1:691.
15. Mollison PL. Bloodtransfusion in clinical medicine. 7th ed. Oxford: Blackwell, 1983.
16. Ouwehand WH. The activity of IgG1 and IgG3 antibodies in immune-mediated destruction of red cells; clinical significance in rhesus haemolytic disease of the newborn [Thesis]. Amsterdam, The Netherlands: University Press, 1984.

Chorionic biopsy in management of severe Kell alloimmunization

F. Rodesch, M.D., M. Lambermont, M.Sc., C. Donner, M.D., Ph. Simon, M.D., F. Romasco, B.S., J. Schwers, M.D., and J. Wybran, M.D.

Brussels, Belgium

Determination of fetal red blood cell Kell antigen is possible on fetal red blood cells obtained at 10 weeks of gestation by chorionic biopsy and may contribute to the management of severe Kell-hemolytic disease. (AM J OBSTET GYNECOL 1987;156:124-5.)

Key words: Chorionic biopsy, Kell antigen, hemolytic disease

As has been shown for Rh_0 (D) immunization,[1] it would be of great help for severely immunized women with anti-Kell antibodies and with a partner heterozygous for Kell antigen to have the fetal Kell antigen status determined in early pregnancy.

A chorionic biopsy was performed before termination of pregnancy. The cell suspension obtained after mincing of the chorionic villi collected by aspiration with a syringe through a Portex catheter reliably contained, as shown by the Kleihauer test, 1% fetal red blood cells among the maternal red blood cells. An immunogold silver staining technique as previously described[2] was used for the demonstration of the Kell antigen. Briefly, this technique was performed on a 2% red blood cell suspension and successively used an anti-Kell serum, a rabbit antihuman immunoglobulin G serum, and a colloidal gold–labeled goat antirabbit serum, followed by silver sensitization. The developed cytocentrifuged preparations were counterstained with standard May-Grünwald Giemsa stain and examined under a light microscope. As many of the fetal red blood cells were erythroblasts, they were easily identified. A positive control consisting of a suspension of 1% Kell-positive red blood cells among Kell-negative red blood cells was observed. The Kell-positive cells were heavily coated with gold-silver particles whereas Kell-negative cells had no such particles on their surfaces. We also demonstrated by this technique Kell antigen on fetal red blood cells obtained by chorionic biopsy before termination of a 9 weeks' gestation from a Kell-positive mother.

From the Unit of Fetal Medicine, Department of Obstetrics and Gynecology, and the Department of Immunology, Hematology and Transfusion, Erasme Hospital and I.R.I.B.H.N., Free University of Brussels.

This work was supported by Fonds de la Recherche Scientifique Medicale.

Received for publication June 20, 1986; accepted July 11, 1986.

Reprint requests: Dr. F. Rodesch, Department of Obstetrics and Gynecology, Erasme Hospital, Route de Lennick, 808, 1070 Brussels, Belgium.

Case report

A 29-year-old woman, gravida 3, para 2, who had never received a blood transfusion, was referred because of fetal death early in the third trimester in her two previous pregnancies. After the second delivery, a Kell hemolytic disease was suggested by a high maternal anti-Kell titer (Coombs 1/1000). The phenotype of the patient and her husband were established, being ccdee, kk and CCDee, Kk, respectively. We discussed with these parents who were facing a pregnancy with a risk of severe Kell-hemolytic disease (the father being heterozygous for the Kell antigen), the possibility of fetal red blood cell Kell antigen determination. Termination of pregnancy if the fetus appeared to be Kell positive was offered as an alternative to difficult and uncertain fetal management. With the immunogold silver staining technique described,[2] we were able to demonstrate that the fetal red blood cells collected at 10 weeks of gestation by chorionic biopsy did not possess the Kell antigen. The maternal anti-Kell titer was high (1/2000). At 24 weeks of gestation a pure fetal blood sample (3 ml) was obtained by direct puncture of the umbilical cord with a 21-gauge needle under ultrasound guidance. The phenotype of the fetal red blood cells was shown by standard agglutination methods to be O, CcDee, kk, thus confirming our previous finding in fetal red blood cells at 10 weeks. The direct Coombs test was negative. A 3760 gm healthy female infant was delivered after spontaneous labor at 38 weeks' gestation. The direct Coombs test was negative and the fetal phenotype O, CcDee, kk was confirmed in the cord blood.

Comment

This is the first report of Kell antigen demonstration on fetal red blood cells obtained by chorionic biopsy in the first trimester of pregnancy. For couples facing a high-risk pregnancy because of Kell hemolytic disease, abortion of a Kell-positive fetus could be an alternative to difficult and uncertain management. On the contrary early demonstration of a kk fetal phenotype would relieve anxiety and prevent unnecessary later diagnostic procedures.

REFERENCES

1. Kanhai HHH, Bennebroek Gravenhorst J, Van't Veer MB, Maas GJ, Beverstock GC, Bernini LF. Chorionic biopsy in management of severe rhesus isoimmunisation. Lancet 1984;1:157-8.
2. Romasco F, Rosenberg J, Wybran J. An immunogold silver staining method for the light microscopical analysis of blood lymphocyte subsets with monoclonal antibodies. Am J Clin Pathol 1985;84:307-16.

Spontaneous rupture of the membranes, functional dystocia, oxytocin treatment, and the route of delivery

Joseph Seitchik, M.D., Alan E. C. Holden, M.A., and Maria Castillo, R.N.
San Antonio, Texas

The details of clinical management were examined in 96 nulliparous patients with functional dystocia who had spontaneous rupture of the membranes before admission and were treated with oxytocin in the first stage of labor. It was our hypothesis that if the allegedly "high" rate of cesarean sections was the result of mediocre or flawed practices, these should be most evident in patients delivered abdominally. A group of 59 patients delivered vaginally were compared with 37 patients delivered by cesarean section. The means of many variables were not statistically different. The cesarean section group was characterized by smaller stature, a lesser cervical dilatation rate both before and after oxytocin administration, a larger maximum oxytocin dose, a longer period of oxytocin administration, more frequent cessation of oxytocin administration or dose reduction because of hypercontractility or an abnormal fetal heart rate or both, and a higher incidence of intra-amniotic infection. We conclude that the decision to perform cesarean section in nulliparous women with functional dystocia arises from disabilities of the patient and not from differences in the application of our management principles, services, or treatments. (Am J Obstet Gynecol 1987;156:125-30.)

Key words: Spontaneous membrane rupture, oxytocin, functional dystocia, cesarean section

In a previous study of patients with intact membranes and functional dystocia, we demonstrated that the application of our diagnostic and therapeutic concepts and techniques was no different in the patients who were delivered by cesarean section or vaginally.[1] We concluded that these modern management principles are not contributors to the increased rate of abdominal delivery. In this study, the same question was asked concerning patients with spontaneous rupture of the membranes before hospital admission and who had spontaneous labor: Were the diagnostic and therapeutic practices different in the groups of patients delivered vaginally and abdominally?

Methods

Study patients were selected in the following manner. During the approximate year from April 1, 1983, to March 10, 1984, 300 consecutive cesarean sections were

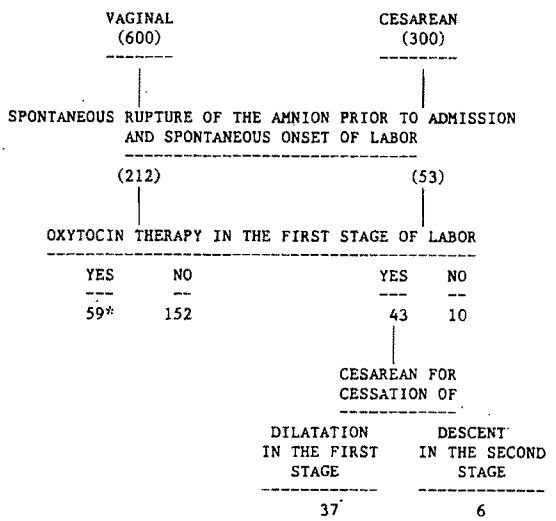


Fig. 1. The plan of the study. Three hundred patients delivered abdominally of a singleton fetus formed the cesarean section group. The first nulliparous patient delivered vaginally of a singleton fetus just before and the first delivered just after the cesarean section formed the vaginal delivery group (N = 600). The 59 patients with spontaneous rupture of the membranes and oxytocin therapy who were delivered vaginally were compared with the 37 patients with failure to achieve complete dilatation and who were delivered abdominally. *Asterisk*, One patient with extensive missing data was excluded.

From the Department of Obstetrics and Gynecology and the Department of Computer Resources, The University of Texas Health Science Center at San Antonio.
Received for publication June 5, 1986; accepted August 14, 1986.
Reprint requests: Dr. Joseph Seitchik, Department of Obstetrics and Gynecology, The University of Texas Health Science Center of San Antonio, 7703 Floyd Curl Dr., San Antonio, TX 78284.

Table I. Data from admission to decision for oxytocin

	Cesarean section	*Vaginal delivery*	*p*
No. of patients	37	59	—
Age <20 yr (%)	62	71	NS
Height <60 inches (%)	41	5	<0.001
Duration of pregnancy			
Preterm (%)	3	15	
Postterm (%)	24	12	
Admission at first labor room visit (%)	87	78	NS
Admission initiated by patient (%)	89	88	NS
Preeclampsia diagnosed at admission and treated with magnesium sulfate (%)	30	14	NS
Patients with no or irregular contractions at admission (%)	32	36	NS
Elapsed time from admission to decision for oxytocin (hr, mean ± SD)	8.2 ± 3.7	6.7 ± 3.8	NS
Cervical dilatation (cm, mean ± SD)			
At admission	2.4 ± 1.4	2.6 ± 1.4	NS
Before oxytocin	3.8 ± 1.6	4.3 ± 1.6	NS
Cervical dilatation rate from admission to last examination before start of oxytocin (cm/hr, mean ± SD)	0.2 ± 0.1	0.3 ± 0.2	<0.01

performed in nulliparous patients with singleton fetuses. The last nulliparous woman who was delivered vaginally just before each cesarean section and the first who was delivered just afterward composed the control group (Fig. 1). There were 265 patients who experienced spontaneous rupture of the membranes and spontaneous onset of labor. Of the 212 who were delivered vaginally, 60 of 212 (28%) required oxytocin. One of the 60 patients had so little information recorded that her data were removed from the vaginal delivery group, with 59 patients remaining in that set. Of the 53 patients who were delivered by cesarean section, 43 of 53 (81%) received oxytocin. Failure to attain complete dilatation was observed in 37 of 43 (86%) and these 37 cases formed the cesarean section group. The Statistical Package for the Social Sciences was used for data analysis,[2] and the statistical methods included χ^2, two-tailed Student's *t* test, one-way analysis of variance, and multiple regression. Faculty members of the Department of Obstetrics and Gynecology and obstetric and family practice residents served as the attending physicians. The decision to use oxytocin was the judgment of senior obstetric residents or faculty; the choice of cesarean section was the prerogative of the faculty; the interim care was provided by junior residents. No specific oxytocin regimen was used; any dosage schedule recommended in textbooks or peer-reviewed journals was acceptable.

Results

The only significant differences at admission between the abdominal and vaginal delivery groups as to the decision to start oxytocin were in the heights of the patients and the mean cervical dilatation rates (Table I). The elapsed mean duration of time from admission to complete dilatation (vaginal group) or to the decision for cesarean section was grossly longer in the group delivered abdominally (Table II). This result obtained because the cesarean section patients averaged 5 more hours (7.8 versus 2.8) from the start of oxytocin to the final dose change. The other time segments of the patients' stay in the labor suite—from admission to the decision for oxytocin, from the decision for oxytocin to its start, or from the last dose change to complete dilatation (vaginal group) or to the decision for cesarean section—were similar. This prolonged period of dose selection in the cesarean section group was associated with a significantly higher mean maximum dose and a greater incidence of stopping or reducing the dose. The rate of incrementation of the oxytocin dose was similar in both groups, as was the frequency of operative delivery associated with abnormal fetal heart rate, birth weight, and 5-minute Apgar score. The incidence of intra-amniotic infection diagnosed during labor was three times greater in the cesarean section group ($p < 0.01$).

Both groups were examined at similar intervals after the start of oxytocin (Table III). The vaginal delivery group manifested a significantly greater mean dilatation rate at each sequential examination, but the large standard deviations indicate that some patients had rapid dilatation and others little, if any. The patients destined to be delivered vaginally had full dilatation at a faster rate than the decision for abdominal delivery was reached in the cesarean section group. Almost two thirds of the vaginal delivery group had full dilatation by an average of 7.3 hours, but the decision for operative delivery had been made by a mean of 8.4 hours in only about one fifth of the cesarean section patients.

Once functional dystocia was diagnosed and the decision to use oxytocin made, the remainder of the labor room stay of the patients undergoing cesarean section was composed mainly of the period from the start of oxytocin to the last dose change (an average of 7.8 of

Table II. Data after decision to use oxytocin

	Cesarean section	*Vaginal delivery*	*p*
No. of patients	37	59	—
Elapsed time (hr, mean ± SD)			
From decision for oxytocin to start of oxytocin	0.7 ± 0.7	0.6 ± 0.5	NS
From start of oxytocin to last dose change	7.8 ± 5.6	2.8 ± 2.8	<0.001
From last dose change to complete dilatation or decision for cesarean section	2.3 ± 2.9	2.7 ± 2.2	NS
From admission to complete dilatation or decision for cesarean section	19.1 ± 6.5	12.7 ± 5.4	<0.001
Cervical dilatation rate (cm/hr, mean ± SD), from decision for oxytocin to complete dilatation or decision for cesarean section	0.2 ± 0.2	1.4 ± 1.0	<0.001
Dose of oxytocin (mU/m)			
Maximum (mean ± SD)	7.8 ± 6.0	4.6 ± 3.5	<0.001
Maximum <10 (%)	92	68	<0.01
Final (mean ± SD)	6.4 ± 5.0	4.3 ± 3.3	<0.02
Oxytocin stopped or dose reduced for abnormal fetal heart rate or hypercontractility (%)	46	19	<0.005
Oxytocin incrementation* rate (%)			NS
None	5	19	
<30 min/mU	51	46	
>29 min/mU	43	36	
Operative delivery associated with abnormal fetal heart rate (%)	14	5	NS
Patients with intra-amniotic infection before delivery (%)	38	12	<0.01
Apgar score <7 at 5 min (%)	0	3	NS
Birth weight (gm, mean ± SD)	3515 ± 538	3329 ± 458	NS

*The mean of the shortest pair of intervals between dose changes or the only interval if only one dose change.

Table III. Cervical dilatation rates observed at six sequential examinations after start of oxytocin

Sequential examination No.	*Patients remaining for study*				*Elapsed time from last examination before start of oxytocin (hr, average ± SD)*			*Cervical dilatation rate (cm/hr, average ± SD)*		
	Cesarean section		*Vaginal delivery*							
	n	%	*n*	%	*Cesarean section*	*Vaginal delivery*	*p*	*Cesarean section*	*Vaginal delivery*	*p*
1	37	100	59	100	2.6 ± 1.4	2.3 ± 3.0	NS	0.3 ± 0.4	0.8 ± 1.1	0.01
2	36	97	56	95	4.1 ± 1.8	3.7 ± 3.1	NS	0.3 ± 0.6	1.6 ± 1.9	<0.001
3	35	95	47	80	5.3 ± 2.0	5.4 ± 1.8	NS	0.5 ± 0.9	1.2 ± 1.2	0.005
4	32	86	40	68	6.7 ± 2.2	6.4 ± 3.8	NS	0.4 ± 0.7	1.3 ± 1.8	0.01
5	30	81	25	36	8.4 ± 2.5	7.3 ± 2.7	NS	0.2 ± 0.6	1.4 ± 1.3	<0.001
6	25	68	19	32	10.2 ± 3.1	9.2 ± 2.8	NS	0.2 ± 0.4	1.4 ± 1.5	<0.001

10.9 hours). It is logical to assume that the major cause of variance in the duration of this "dose-finding" period would be the maximum dose of oxytocin administered. When the data for this variable were segmented into four class intervals (<x − 1 SD, x − 1 SD to x, x to x + 1 SD, >x + 1 SD), one-way analysis of variance demonstrated that the maximum dose of oxytocin was the only variable with a value that fluctuated significantly with the duration of the several class intervals of the "dose-finding" period (Table IV). Multiple regression analysis demonstrated that 77% ($R^2 = 0.77$, $p < 0.001$) of the "dose-finding" time variance can be accounted for. Fluctuations in the values for the maximum dose were responsible for 72% of the explained variance; the presence of preeclampsia and the use of magnesium sulfate and stopping or reducing the dose played minor additional roles. With either method of analysis, it is clear that the maximum dose of oxytocin was the major determinant of the length of the "dose-finding" period, which, in turn, was responsible for the duration of the patients' labor room stay once the diagnosis of functional dystocia and the decision to use oxytocin were made.

In contrast, for the vaginal delivery patients, the "dose-finding" period occupied less than half of the patients' time in the labor suite from the decision to use oxytocin to complete dilatation (an average of 2.8 of 6.1 hours). Segmenting the data by the same four class intervals of the "dose-finding" period and use of one-way analysis of variance revealed that the values

Table IV. Values of different variables when data are segregated by class intervals of time from start of oxytocin to last dose change and route of delivery

Start of oxytocin to final dose change (hr)	*No. of patients*	*Cervical dilatation, start of oxytocin (cm, mean ± SD)*	*Maximum dose of oxytocin (mU/m, mean ± SD)*	*Elapsed time to complete dilatation or decison for cesarean section* (hr, mean ± SD)*	*Time to start of oxytocin* (hr, mean ± SD)*	*Last dose change to complete dilatation or decision for cesarean section (hr, mean ± SD)*
Cesarean section						
All	37	3.8 ± 1.6	7.8 ± 6.0	10.9 ± 5.6	0.7 ± 0.7	2.3 ± 2.9
0.0-2.1	8	3.8 ± 1.4	2.9 ± 1.0†	6.4 ± 4.6†	1.0 ± 1.3	4.5 ± 4.1
2.2-7.7	13	4.6 ± 2.0	5.2 ± 1.9†	7.6 ± 3.1†	0.7 ± 0.6	1.7 ± 2.3
7.8-13.0	10	3.1 ± 0.9	10.2 ± 4.0‡	14.2 ± 2.7‡	0.8 ± 0.5	2.0 ± 2.5
>13.0	6	3.6 ± 1.0	16.0 ± 8.4§	18.5 ± 3.1§	0.5 ± 0.5	1.4 ± 1.2
p‖		NS	<0.0001	<0.0001	NS	NS
Vaginal delivery						
All	59	4.3 ± 1.6	4.6 ± 3.5	6.1 ± 3.9	0.6 ± 0.5	2.7 ± 2.2
0.0	10	5.1 ± 2.2†	1.1 ± 0.3§	3.9 ± 2.0†	0.6 ± 0.5	3.3 ± 1.8
0.1-2.8	27	4.6 ± 1.6†	3.9 ± 1.8‡	3.9 ± 1.7†	0.6 ± 0.5	2.0 ± 1.3
2.9-5.6	11	4.1 ± 1.1†‡	6.9 ± 4.6†	8.2 ± 4.2‡	0.4 ± 0.3	3.7 ± 3.9
>5.6	11	3.0 ± 1.0‡	7.0 ± 4.1†	11.4 ± 2.6§	0.9 ± 0.5	2.9 ± 1.4
p‖		0.014	<0.0001	<0.0001	NS	NS

*From the time of the last examination before the start of oxytocin.

†The Student–Newman-Keuls test indicates that this (these) group (groups) differs from the others at the 0.05 level.

‡The Student–Newman-Keuls test indicates that this (these) group (groups) differs from the others at the 0.05 level.

§The Student–Newman-Keuls test indicates that this (these) group (groups) differs from the others at the 0.05 level.

‖The f test probability.

Table V. Comparison of data from patients in spontaneous labor who received oxytocin segregated by amniotomy or spontaneous rupture of the amnion and by route of delivery

	Vaginal delivery			*Cesarean section*		
	Amniotomy	*Spontaneous rupture of membranes*	*p*	*Amniotomy*	*Spontaneous rupture of membranes*	*p*
No. of patients	68	59		33	37	
Preterm (%)	8	15	NS	3	3	NS
Preeclampsia and magnesium sulfate (%)	32	14	0.02	48	30	0.03
Contractions at admission (%)						
<every 6 min	78	58		70	54	
>every 5 min, "irregular," or "none"	22	42		30	46	
Cervical dilatation (cm, mean ± SD)						
At admission	3.8 ± 1.1	2.6 ± 1.4	<0.001	3.3 ± 1.5	2.4 ± 1.4	0.01
At decision for oxytocin	5.3 ± 1.7	4.3 ± 1.6	0.001	4.6 ± 1.7	3.8 ± 1.7	0.05
At decision for cesarean section	—	—		7.1 ± 1.3	6.2 ± 1.8	0.05
Change in cervical dilatation from admission to decision for oxytocin <1.0 cm (%)	25	27	NS	24	24	NS
Elapsed time (hr, mean ± SD)						
Admission to decision for oxytocin	4.5 ± 2.5	6.7 ± 3.8	<0.001	7.3 ± 4.0	8.2 ± 3.7	NS
Start of oxytocin to last dose change	2.7 ± 3.3	2.8 ± 2.8	NS	6.2 ± 4.7	7.8 ± 5.6	NS
Last dose change to complete dilatation or decision for cesarean section	2.2 ± 2.0	2.7 ± 2.2	NS	2.3 ± 2.5	2.3 ± 2.9	NS
Admission to complete dilatation or decision for cesarean section	10.8 ± 3.9	12.7 ± 5.4	0.02	16.0 ± 4.3	19.1 ± 6.5	0.03
Maximum dose of oxytocin (mU/m, mean ± SD)	4.1 ± 3.8	4.6 ± 3.5	NS	6.1 ± 6.0	7.8 ± 6.0	NS
Dose of oxytocin ever stopped or dose reduced (%)	21	22	NS	39	49	NS
Intra-amniotic infection (%)	7	12	NS	3	38	<0.001

Oxytocin stopped or dose reduced		*Magnesium sulfate given for pregnancy-induced hypertension*	
n	%	*n*	%
18	49	11	30
2	25	1	13
5	39	3	23
7	70	4	40
4	67	3	50
NS		NS	
13	22	8	14
1	10†	3	30
2	7†	1	4
4	36†‡	3	27
6	55‡	1	9
0.0023		NS	

for the maximum dose of oxytocin, stopping or reducing the dose, and cervical dilatation fluctuated significantly (Table IV). Multiple regression demonstrated that 59% of the variance in the "dose-finding" period could be explained ($r = 0.59$, $p < 0.001$). Stopping or reducing the dose and the interactions of that variable accounted for 58% of the explained variance, whereas the maximum dose of oxytocin and its interactions explained 35%. We interpret these data to suggest that when the range of maximum doses for most patients was small, as in the vaginal delivery group, factors other than dose tended to be more important in the determination of the length of the "dose-finding" period.

Comment

Our observations indicated that the need for abdominal delivery was not an artifact created by our practices. Patients destined for cesarean section were given larger doses of oxytocin during longer periods of time and, once the selected maximum dose was administered, were observed for a period of time similar to that required by the vaginal delivery patients to reach full dilatation. Smaller stature and intra-amniotic infection diagnosed before delivery occurred more frequently in the cesarean section group.

Comparison of patients with functional dystocia admitted with ruptured membranes with those experiencing amniotomy suggests that, in our experience, the groups were not identical (Table V). Regardless of mode of delivery, at admission the group with spontaneous rupture of the membranes had fewer patients with preeclampsia treated with magnesium sulfate but more having poor contractile activity and less cervical dilatation and longer stays in the labor suite until complete dilatation or the decision for cesarean section. The difference in the mean duration of the labor room experience for patients delivered vaginally was the result of a significantly longer time for the patients with spontaneous rupture of the membranes between admission and the decision to use oxytocin ($p < 0.001$). The combinations of lesser cervical dilatation and contraction frequency and fewer patients with preeclampsia probably account for this difference.

Little cervical dilatation and infrequent or no contractions always engender the question, "Is the patient in labor?" In nulliparous women, we can avoid induction of labor with its attendant high cesarean section rate by observation of the patient during a period of time. In the absence of any compelling indication for delivery, such as preeclampsia, we found no contraindication to waiting for contractions to appear in patients who had none or for contractions to improve, persist, or disappear in those who had them infrequently. On our service, the rate of cesarean section because of failure to achieve complete dilatation among nulliparous women with spontaneous labor and oxytocin therapy for functional dystocia is 13% for those with amniotomy and 16% for those with spontaneous rupture of the membranes, a nonsignificant difference. This policy, which insists that contractions be present and persist before the diagnosis of labor is accepted, has not contributed to the frequency of abdominal delivery.

Alternatively it seems unreasonable to insist that the diagnosis of labor requires some observed change in cervical dilatation once the membranes are ruptured, spontaneously or artificially. Regardless of the mechanism of rupture of the amnion or the route of delivery, approximately 25% of each group failed to demonstrate a change in cervical dilatation of ≥ 1 cm from admission to the decision for oxytocin (Table V). Our avoidance of the requirement that some significant change in dilatation occur in each and every patient before the diagnosis of labor and functional dystocia was entertained did not appear to contribute to the frequency of cesarean section. If we were incorrect in this judgment, then the percentage of patients with no cervical dilatation from admission to the decision to use oxytocin would have been significantly greater in the patients delivered abdominally.

It is common wisdom that the longer the labor, the higher the incidence of intra-amniotic infection. For the patients with spontaneous rupture of the membranes, the mean duration from admission to the decision for cesarean section was 19.1 hours. The diagnosis of intra-amniotic infection before delivery was made in 14 (38%) patients and was present in only one case at admission, becoming manifest in the other 13 during labor. What potential do we have for reducing the length of labor? We suggest two possibilities. The mean time from admission to the use of oxytocin might be reduced if, once the diagnosis of labor is made, we

insist that each patient manifest a detectable change in dilatation in each 2-hour period.[1] If not, appropriate therapy should be accomplished promptly. This management principle requires omitting the concept of a latent phase of first-stage labor from our evaluation of these patients. The second opportunity may exist in the "dose-finding" period. The use of regimens that rapidly increment the dose and their resultant large maximum doses can produce hypercontractility and deviations from fetal heart rate norms. These events, most evident in patients monitored by electronic devices, cause the oxytocin to be stopped or the dose reduced frequently in patients destined for cesarean section. As a result, the process of finding the needed dose becomes inefficient. The use of regimens designed from the knowledge that (1) any specific dose of oxytocin requires about 40 minutes to reach a steady-state plasma concentration and (2) 70% to 75% of the patients with functional dystocia will need a dose of ≤4 mU/m and 95%, ≤9 mU/m may reduce the length of the "dose-finding" time.[4,5]

REFERENCES

1. Seitchik J, Holden AEC, Castillo M. Amniotomy and oxytocin treatment of functional dystocia and route of delivery. AM J OBSTET GYNECOL 1986;155:585.
2. Nie NH, ed. SPSS. Statistical package for the social sciences. 2d ed. New York: McGraw-Hill, 1975.
3. Seitchik J, Holden AEC, Castillo M. Amniotomy and the use of oxytocin in labor in nulliparous women. AM J OBSTET GYNECOL 1985;153:848.
4. Seitchik J, Castillo M. Oxytocin augmentation of dysfunctional labor. I. Clinical data. AM J OBSTET GYNECOL 1982; 144:899.
5. Seitchik J, Amico J, Robinson AG, Castillo M. Oxytocin augmentation of dysfunctional labor. IV. Oxytocin pharmacokinetics. AM J OBSTET GYNECOL 1984;150:225.

Polyhydramnios and elevated intrauterine pressure during amnioinfusion

Bannie L. Tabor, M.D., and Jeffrey A. Maier, M.D.
Torrance, California

Amnioinfusion has been demonstrated to be useful in relieving and preventing repetitive variable decelerations. There have been no reported complications. This case illustrates the potential for iatrogenic polyhydramnios and elevated intrauterine pressure during amnioinfusion. (AM J OBSTET GYNECOL 1987; 156:130-1.)

Key words: Amnioinfusion, polyhydramnios, elevated intrauterine pressure

Saline amnioinfusion has been demonstrated to be effective in preventing and relieving repetitive variable decelerations. This results in a lower cesarean section rate for fetal distress in nulliparous patients[1] and in an improved metabolic state in infants of patients with preterm premature rupture of the membranes.[2] There have been no reported complications with this procedure.

We report a case that shows that in certain situations the procedure is not without risk.

From the Division of Obstetrics, Department of Obstetrics and Gynecology, Harbor/University of California, Los Angeles, Medical Center.
Received for publication June 5, 1986; accepted July 9, 1986.
Reprint requests: Bannie L. Tabor, M.D., Department of Obstetrics and Gynecology, Harbor/UCLA Medical Center, 1000 W. Carson St., Torrance, CA 90509.

Case report

A 21-year-old woman, gravida 3, para 1, at 34⅘ weeks' gestation, presented with a 4½-hour history of spontaneous rupture of the membranes without uterine contractions. A sterile speculum examination confirmed ruptured membranes. The cervix appeared long and closed. Adequate amniotic fluid was noted on ultrasound examination. An oxytocin induction of labor was started. Amniotic fluid continued to leak during the next 24 hours as the cervical examination revealed progression to 2 cm dilation, 50% effacement, and −1 station. A repeat ultrasound examination showed decreased amniotic fluid, the largest pocket measuring <2 cm vertically. The patient was randomized to receive amnioinfusion in an ongoing approved human subjects protocol.

The intrauterine pressure was 10 mm Hg before the amnioinfusion. A total of 600 ml of normal saline solution was infused during 1 hour by an intermittent infusion pump through an attachment to the internal

uterine catheter. The resting intrauterine pressure increased to 15 mm Hg. A maintenance rate of 180 ml/hr was then begun; 2700 ml of fluid was infused during the next 15 hours accompanied by an increase in the uterine pressure to 20 mm Hg. Modest vaginal leakage was noted.

An additional liter of fluid was infused during the next 5 hours without vaginal leakage. The intrauterine baseline pressure gradually increased to 50 mm Hg. The fetus then had two prolonged decelerations, followed by a 6-minute bradycardiac episode to 70 bpm. This was relieved by stopping the oxytocin, changing the patient's position, and administering tocolysis with terbutaline, 0.25 mg subcutaneously. The patient complained of constant abdominal pain. On examination the uterus was very tense and tender. The cervix was 2 cm dilated, 70% effaced, and at −1 station. The amnioinfusion was halted. The resting pressure remained elevated at 45 mm Hg. Ultrasound examination revealed marked polyhydramnios, the fluid pocket measuring >10 cm vertically. Uterine contractions returned and were associated with repetitive moderate variable decelerations. These were relieved by draining 150 ml of amniotic fluid; an additional 750 ml of fluid was drained before the pressure returned to normal (15 to 20 mm Hg). Thereafter the fetal heart rate was 155 bpm with good variability and reactivity. The uterus was soft and nontender between contractions.

Oxytocin was restarted, and the patient was ultimately delivered of a 2350 gm male infant with Apgar scores of 7 at 1 minute and 8 at 5 minutes. The umbilical arterial pH was 7.23 and the venous pH was 7.35. There was no nuchal cord or any other cord abnormalities. Before delivery the patient developed amnionitis and was placed on a regimen of ampicillin, which was continued post partum. Her postpartum course was uncomplicated. The infant initially exhibited mild diffuse hypotonia, which spontaneously resolved. He was treated with antibiotics until all cultures were returned negative. A subgaleal hematoma was noted and was felt to be due to the prolonged course of labor. He was discharged on day 7, doing well and without sequelae.

Comment

In previously reported studies there has been no mention of significantly increased intrauterine pressure or polyhydramnios with amnioinfusion. Miyazaki and Nevarez[1] reported an increase in the resting pressure to 35 to 40 mm Hg during the gravity-driven bolus infusion, which was artifactual. In a personal communication, Nageotte also related an increase in baseline pressure during prophylactic amnioinfusion. Our protocol is similar to Nageotte's, in that the fluid is actively infused by a mechanical pump. Due to the pulsatile action of the pump, the tokodynamometer records a compact sine wave because of the increased pressure in the catheter as the pump infuses. The true intrauterine pressure coincides with the wave trough and can be confirmed by closing the connection between the pump and the intrauterine catheter.

With both techniques fluid is infused under pressure, be it mechanical pressure from the pump or hydrostatic pressure from the column of water in the gravity infusion system. This has little deleterious effect in most cases as the fluid is infused into an open system. As labor progresses, there is space between the dilating cervix and the fetal presenting part, which allows fluid and pressure to escape.

In the reported case, the fetal head was firmly applied to the cervix, thus creating a closed system. The pump continued to infuse fluid, resulting in polyhydramnios and eventually a significant increase in intrauterine pressure. This could arise with the use of either method as both have the potential for infusing fluid under pressure. The precise pathophysiologic mechanism of the associated fetal heart rate decelerations is unclear. We speculate that the increased intrauterine pressure prevented adequate uteroplacental perfusion.

This situation can be avoided by carefully monitoring the true intrauterine pressure during amnioinfusion and regularly assessing the amount of amniotic fluid by ultrasound. Maintaining these within normal limits should prevent fetal compromise in this otherwise safe procedure.

REFERENCES

1. Miyazaki FS, Nevarez F. Saline amnioinfusion for relief of repetitive variable decelerations: a prospective randomized study. Am J Obstet Gynecol 1985;153:301-6.
2. Nageotte MP, Freeman RK, Garite TJ, Dochester W. Prophylactic intrapartum amnioinfusion in patients with preterm premature rupture of membranes. Am J Obstet Gynecol 1985;153:557-61.

Screening for gestational diabetes: One-hour carbohydrate tolerance test performed by a virtually tasteless polymer of glucose

E. Albert Reece, M.D., Theodore Holford, Ph.D., Susan Tuck, M.D., Mitzi Bargar, R.N., Theresa O'Connor, M.P.H., and John C. Hobbins, M.D.

New Haven, Connecticut

Although the 1-hour 50 gm blood glucose screening test is an effective way of detecting diabetes in pregnancy, the taste of available glucose drinks often creates gastrointestinal symptoms and leads to refusal of the patient to be tested. The efficacy of a virtually tasteless glucose polymer in testing carbohydrate tolerance in pregnancy was determined. Sixty-one pregnant patients undergoing screening for gestational diabetes underwent a 1-hour carbohydrate tolerance test of both glucose and a glucose polymer within 3 days of each other. Analysis of the data revealed a high degree of agreement between the results of the 1-hour carbohydrate tolerance test ($\kappa = 0.62$, $p < 0.0001$). These data suggest that glucose polymer can be used effectively in screening for gestational diabetes. (AM J OBSTET GYNECOL 1987;156:132-4.)

Key words: Carbohydrate tolerance, Polycose

It is well established that pregnancy has a diabetogenic effect, and the glucose intolerance that develops in pregnancy can be associated with an increase in perinatal mortality.[1, 2] The oral glucose tolerance test has become the recommended method to assess carbohydrate metabolism in pregnancy.[3-6] O'Sullivan et al.[5] have demonstrated that a 1-hour 50 gm blood glucose screening test followed by a 3-hour glucose tolerance test, performed in those patients with a blood glucose level exceeding 130 mg/dl of whole blood, is an effective way of identifying potentially diabetic patients. However, glucose drinks currently available are often associated with nausea, vomiting, abdominal bloating, or even headache.[6] Noncompliance and vomiting will render these blood studies meaningless.

Glucose polymer (Polycose*) is a commercially available, inexpensive glucose saccharide mixture that is virtually tasteless and contains 3% glucose, 7% maltose, 5% maltotriose, and 85% polysaccharides of 4 to 15 (mean = 5) glucose units.[7] Its osmotic load is one fifth that of glucose and has been reported to be associated with less gastrointestinal symptoms.[7] The purpose of this study is to determine whether this glucose polymer could be effectively used in a 1-hour carbohydrate tolerance test to screen for gestational diabetes.

From the Diabetes-in-Pregnancy Study Unit of the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, and the Biostatistic Section of the Department of Epidemiology and Public Health, Yale University School of Medicine.

Submitted for publication January 13, 1986; revised May 28, 1986; accepted July 25, 1986.

Reprint requests: E. Albert Reece, M.D., Yale University School of Medicine, Department of Obstetrics and Gynecology, 333 Cedar St., P. O. Box 3333, New Haven, CT 06510-8063.

*Ross Laboratories, Div. Abbott Laboratories, Columbus, OH 43216.

Patients and methods

Patients undergoing diabetes screening were invited to participate in a study where glucose was compared with a glucose polymer in the performance of a carbohydrate tolerance test. Patients were instructed to maintain a 300 gm carbohydrate-enriched diet for 2 to 3 days before the study in order to maximize and standardize the carbohydrate tolerance tests.[8] Patients fasted overnight, and on the morning of the test whole blood determinations of fasting blood sugar were obtained, followed by the ingestion of a 50 gm drink of glucose polymer. A blood glucose level was obtained 1 hour later. Glucose determination was performed by the enzymatic reaction with the use of glucose oxidase as described elsewhere.[9, 10] A repeat test with the standard glucose drink was performed in all patients within 3 days of the first study. Patients were asked to comment on the taste and to report any symptoms, including nausea or vomiting, that they experienced. Patients were not charged for these tests; however, the approximate cost of a 50 gm Polycose drink was 35 cents, compared with the $2.50 cost of a standard 50 gm glucose drink. Normal values used for both tests were those reported by O'Sullivan et al.[5] for glucose. Patients underwent a 3-hour glucose tolerance test if at least one study (glucose or Polycose) was abnormal. In order to determine whether the two test results were equivalent, the data were analyzed with the use of the κ statistic, $\kappa = Po-Pc/1-Pc$, where Po = proportion of observed agreement and Pc = overall proportion of

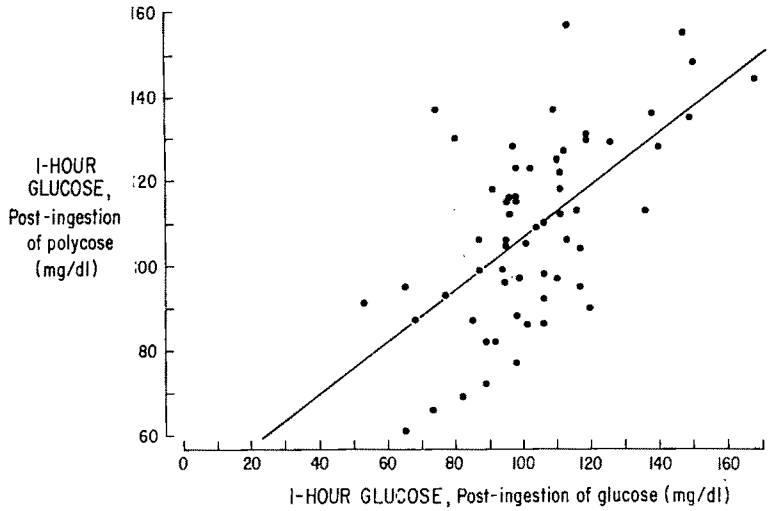


Fig. 1. Scatter plot of 1-hour glucose values after ingestion of glucose polymer (y axis) and glucose (x axis).

Table I. Results of 1-hour carbohydrate tolerance test

Result of glucose test	*Results of Polycose test*			
	Negative		*Positive*	
	n	%	*n*	%
Negative	51	83.6	3	4.9
Positive	2	3.3	5	8.2

$\kappa = 0.62$; $p < 0.0001$.

agreement expected by chance alone. The κ statistic is a measure of the amount of agreement between the two tests beyond that expected by chance alone. A κ statistic <0 indicates less than chance agreement; $\kappa = 0$ indicates just chance agreement; $\kappa > 0$ indicates more than chance agreement; and $\kappa = 1$ indicates perfect agreement.[6]

Results

Sixty-one patients underwent a 1-hour carbohydrate tolerance test with both Polycose and glucose. Of these, 51 (83.6%) had normal results in both tests, five (8.2%) were abnormal in both studies, and in five patients (8.2%) one of the two studies was abnormal (Table I). Although the mean blood glucose levels at fasting in both the Polycose and glucose groups were similar to each other, at 1 hour after Polycose ingestion the blood glucose level was consistently above that observed after the glucose drink but remained well within the normal range (<120 mg/dl) (Table II). Since the false negative rate for a 1-hour glucose tolerance test is <1%, a 3-hour glucose tolerance test with the 100 gm glucose load was performed only in those patients having at least one abnormal study. The normal glucose determinations used were those outlined by O'Sullivan and Mahan.[4] The 3-hour glucose tolerance test was negative for three patients with positive Polycose and negative glucose results, and negative for two patients with negative Polycose and positive glucose results. Of the five patients with positive Polycose and positive glucose results, the 3-hour glucose tolerance test results were positive in two and negative in three. Analysis of the data revealed a high level of agreement between the results of the 1-hour carbohydrate tolerance tests ($\kappa = 0.62$; $p < 0.0001$). Fig. 1 shows a scatter plot of the 1-hour blood glucose levels for the two tests. In addition, patients expressed a greater preference for the Polycose drink than for the glucose. Unfortunately, many patients failed to complete the questionnaire completely. However, about 40% of patients experienced nausea and/or vomiting after glucose compared with about 10% after Polycose ingestion.

Table II. Blood glucose determinations of both Polycose and glucose tolerance tests

Test	*Fasting blood glucose (mg/dl, mean ± SE)*	*Blood glucose 1 hr after ingestion (mg/dl, mean ± SE)*
Polycose	66.67 ± 12.56	108.7 ± 21.9
Glucose	68.63 ± 10.36	103.4 ± 21.9

Comment

The results of this study demonstrate that carbohydrate tolerance testing can be satisfactorily performed by a glucose polymer, which is less expensive and preferred over glucose by patients. The high patient acceptance rate in our study is in agreement with a prior

report by Court et al.[7] Those investigators calculated the area under the curve (incremental area) for glucose and Polycose to determine the reproducibility of the tolerance tests. The more similar the incremental areas of paired tests were with the use of the same agent, the more reproducible the test was considered to be. The correlation between incremental areas of the paired tolerance test was not significant for glucose but was significant for glucose polymer ($p < 0.001$), thus suggesting a greater reproducibility for Polycose than glucose.[7] In our study, although 3-hour glucose tolerance tests were not done in all patients, the high degree of correlation between the 1-hour Polycose and glucose tolerance tests suggests similar true and false negative and true and false positive rates. O'Sullivan et al.[4,5] have shown that the 1-hour glucose tolerance test has a false negative rate of <1% and a false positive rate of 86%. Our studies indicate that similar rates may be presumed for the 1-hour Polycose tolerance test.

The normal metabolic changes of pregnancy produce a "diabetogenic effect," and the incidence of impaired glucose tolerance during pregnancy is about 2% to 3%.[11-13] Prospective studies have shown that perinatal morbidity and mortality are higher in women with gestational diabetes than in randomly selected control patients.[8, 11, 12] Since improved detection of gestational diabetes and more aggressive treatment have resulted in improved rates of perinatal morbidity and mortality, it is extremely important that a screening test for gestational diabetes should not only be sensitive but be acceptable to patients, so that maximal compliance can be achieved. Hoet and Beard[14] compared various screening tests in terms of sensitivity and cost and concluded that the O'Sullivan and Mahan 1-hour glucose tolerance test has the advantage of high sensitivity, acceptable specificity, and low cost. Although the advantages of this test are self-evident, the disadvantages include its taste, which often creates gastrointestinal symptoms as well as refusal of the patient to be tested.

Our comparison of carbohydrate tolerance tests with the use of both glucose and glucose polymer demonstrates striking similarities in test results but disparity in patient satisfaction and tolerance. These data suggest that glucose polymer is preferable to glucose in a 1-hour carbohydrate tolerance test to screen for gestational diabetes.

We wish to thank Nancy Nickless, R.N., Nina Tiglio, R.D., M.P.H., and Lyn Oehler, M.S., R.D., for their assistance in this study.

REFERENCES

1. O'Sullivan JB, Charles D, Mahan C, et al. Gestational diabetes and perinatal mortality rate. Am J Obstet Gynecol 1973;116:901.
2. Dandrow RV, O'Sullivan JB. Obstetric hazards of gestational diabetes. Am J Obstet Gynecol 1966;96:1144.
3. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979;28:1039.
4. O'Sullivan JB, Mahan CM. Criteria for the oral glucose tolerance test in pregnancy. Diabetes 1964;13:278.
5. O'Sullivan JB, Mahan CM, Charles D, et al. Screening criteria for high-risk gestational diabetic patients. Am J Obstet Gynecol 1973;116:895.
6. Fleiss JL. Statistical methods for rates and proportions. New York: John Wiley & Sons, 1972.
7. Court DJ, Stone PR, Killip M. Comparison of glucose and a glucose polymer for testing oral carbohydrate tolerance in pregnancy. Obstet Gynecol 1984;64:251.
8. Stallone LA, Zeil HK. Management of gestational diabetes. Am J Obstet Gynecol 1974;119:1091.
9. Kadish AH, Little RL, Sternberg JC. A new and rapid method for determination of glucose by measurement of rates of oxygen consumption. Chem 1968;21:116.
10. Fischl J, Federman D, Talmon N. Preparation of a modified glucose oxidase reagent for the polarographic determination of glucose with the Beckman "glucose analyzer." Clin Chem 1975;21:760.
11. Hollingsworth DR, Cousins L. Endocrine and metabolic disorders. In: Maternal-fetal medicine. Principles and practice. Philadelphia: WB Saunders, 1984:844-6.
12. Gillmer MDG, Oakley NW, Beard RW, et al. Screening for diabetes during pregnancy. Br J Obstet Gynaecol 1980;87:377.
13. Lavin JP, Barden TP, Miodovnik M. Clinical experience with a screening program for gestational diabetes. Am J Obstet Gynecol 1981;141:491.
14. Hoet JJ, Beard RW. Clinical perspective in the care of the pregnant diabetic patient, In: Pregnancy metabolism. Diabetes and the fetus. Amsterdam: Excerpta Medica, 1979. (Ciba Foundation 63, new series).

POSTGRADUATE OBSTETRICS & GYNECOLOGY

A BIWEEKLY REVIEW OF CLINICAL OBSTETRICS AND GYNECOLOGY

litors

itor-in-Chief
)WARD E. WALLACH, M.D.

sociate Editor
)GER D. KEMPERS, M.D.

sociate Editor
)NALD J. BOLOGNESE, M.D.

litorial Board

ERBERT J. BUCHSBAUM, M.D.
HARLES B. HAMMOND, M.D.
ATHAN KASE, M.D.
)HN J. MIKUTA, M.D.
NNIFER R. NIEBYL, M.D.
)HN T. QUEENAN, M.D.
CHARD H. SCHWARZ, M.D.
:ON SPEROFF, M.D.

A CONTINUING AMA CATEGORY I MEDICAL EDUCATION PROGRAM

Postgraduate Obstetrics and Gynecology is a biweekly publication consisting of 26 lessons per year. Every two weeks during the calendar year each subscriber receives a state-of-the-art program which contains an original article focusing on a basic facet or contemporary problem and a self-test. Each article is prepared by an authority from among our distinguished editorial board or by a guest author selected by our editors. The articles are timely, authoritative, concise, and supplemented by visuals to provide clear illustrations of important details.

Each program concludes with a series of multiple choice questions which crystallize the most significant points of the article. The correct responses for each lesson are contained in the subsequent issue.

Accreditation—As an organization for continuing medical education, the University of Pennsylvania School of Medicine designates this continuing medical education activity as meeting the criteria for 1 credit hour per biweekly issue in Category 1 for Education Materials for the Physician's Recognition Award of the American Medical Association provided it has been completed according to instructions. This course has also been approved by the Americal College of Obstetricians and Gynecologists for 1 cognate per biweekly issue formal learning, as well as by the Committee on Continuing Education of the American Osteopathic Association for 1 credit hour per biweekly issue in Category 2B.

Each paid subscription is entitled to 26 CME credits and written CME certification is available upon request.

Please enter my subscription for one year (26 issues) of **Postgraduate Obstetrics & Gynecology.** I understand that my subscription will start with the issue following receipt of my payment ($160.00).

½ year subscription available—$90 for 13 issues.

(*Overseas orders* please add $35.00 for Air Mail orders.)

(check one)
☐ check enclosed
☐ bill me

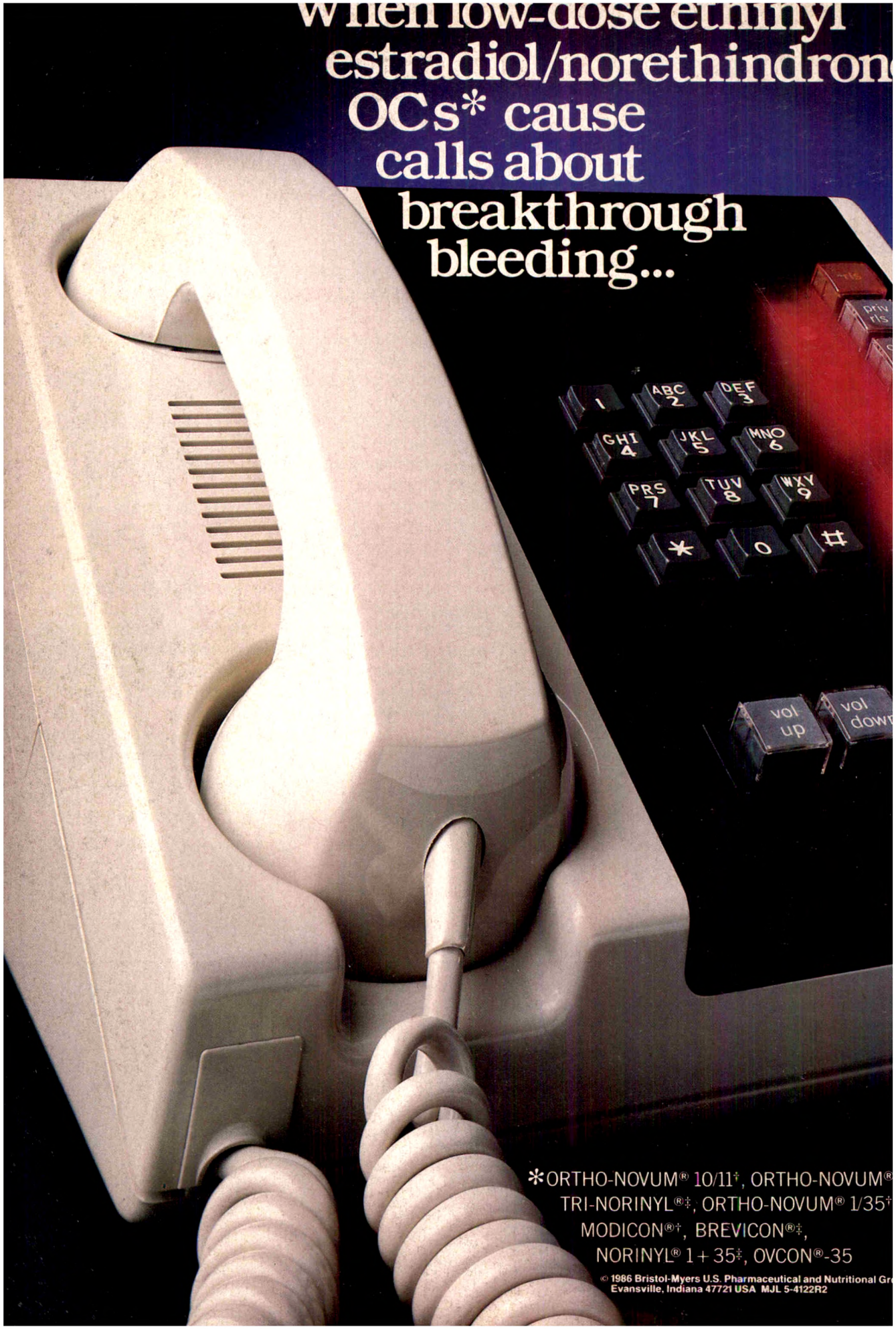
Mail to:
POBG
P.O. Box 23263
Baltimore, MD 21203-9990

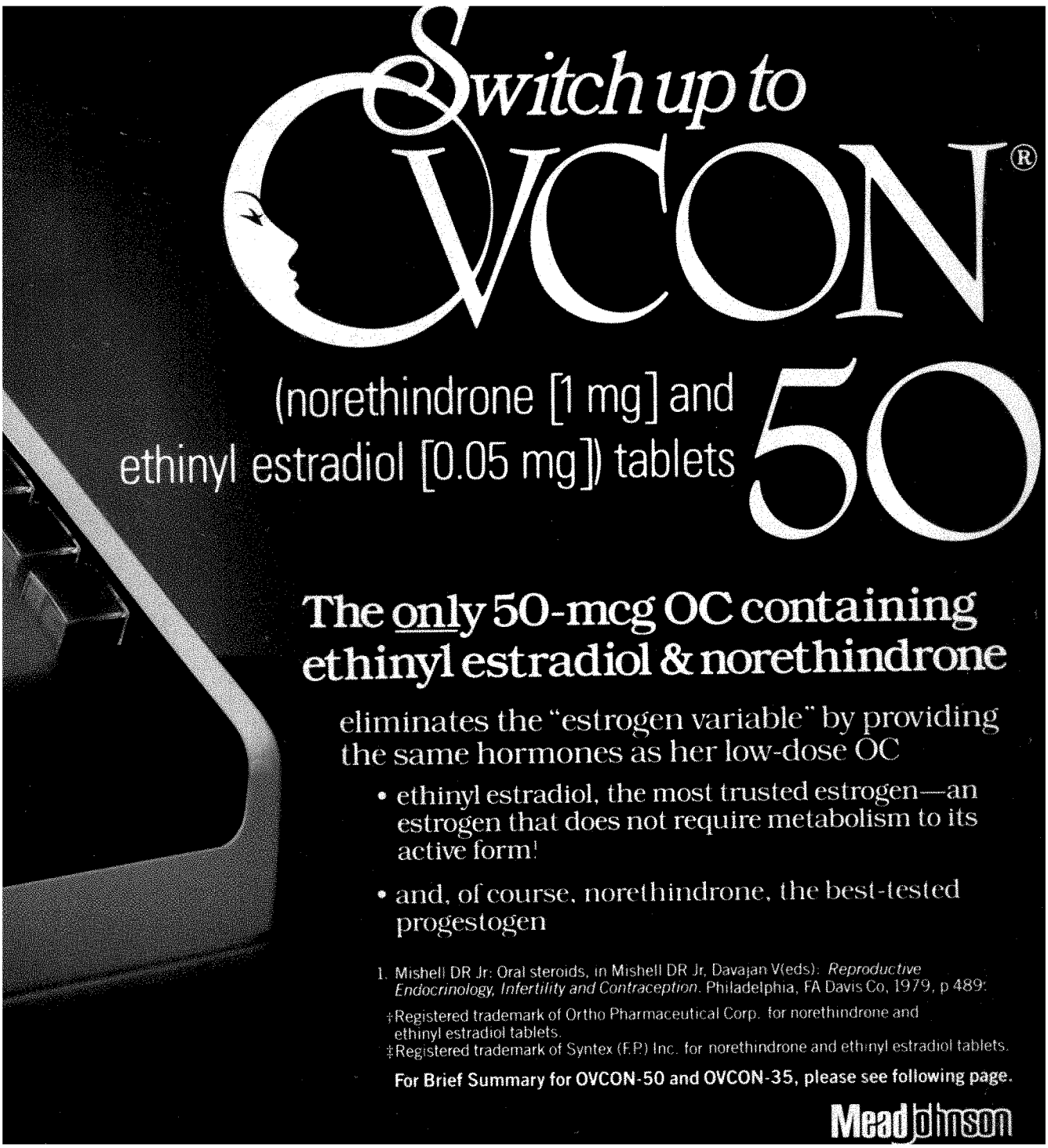
Name _____

Address _____

City _____ State _____ Zip _____

Postgraduate Obstetrics and Gynecology, Inc., Attn: Diane Kitko, Post Office Box 23263, Baltimore, MD 21203-9990, Telephone: 1-(301) 955-7822.


When low-dose ethinyl estradiol/norethindron
OCs* cause calls about breakthrough bleeding...
1
ABC 2
DEF 3
GHI 4
JKL 5
MNO 6
PRS 7
TUV 8
WXY 9
*
0
#
vol up
vol down
*ORTHO-NOVUM® 10/11†, ORTHO-NOVUM® TRI-NORINYL®‡, ORTHO-NOVUM® 1/35†
MODICON®†, BREVICON®‡,
NORINYL® 1+35‡, OVCON®-35
© 1986 Bristol-Myers U.S. Pharmaceutical and Nutritional Gr
Evansville, Indiana 47721 USA MJL 5-4122R2


Switch up to
OVCON®
50
(norethindrone [1 mg] and
ethinyl estradiol [0.05 mg]) tablets
The only 50-mcg OC containing
ethinyl estradiol & norethindrone
eliminates the "estrogen variable" by providing
the same hormones as her low-dose OC
• ethinyl estradiol, the most trusted estrogen—an estrogen that does not require metabolism to its active form!
• and, of course, norethindrone, the best-tested progestogen
1. Mishell DR Jr: Oral steroids, in Mishell DR Jr, Davajan V(eds): Reproductive Endocrinology, Infertility and Contraception. Philadelphia, FA Davis Co, 1979, p 489.
†Registered trademark of Ortho Pharmaceutical Corp. for norethindrone and ethinyl estradiol tablets.
‡Registered trademark of Syntex (F.P.) Inc. for norethindrone and ethinyl estradiol tablets.
For Brief Summary for OVCON-50 and OVCON-35, please see following page.
Mead Johnson

OVCON®-50

(norethindrone [1 mg] and ethinyl estradiol [0.05 mg]) tablets

OVCON®-35

(norethindrone [0.4 mg] and ethinyl estradiol [0.035 mg]) tablets

Indications and Usage: Ovcon is indicated for the prevention of pregnancy. Some combination products containing 35 mcg. or less of ethinyl estradiol report slightly higher pregnancy rates than are reported for the higher dose combination products.

Dose-related Risk of Thromboembolism from Oral Contraceptives: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing 50 mcg. or less of estrogen.

Contraindications: Oral contraceptives should not be used in women with any of the following conditions: 1) Thrombophlebitis or thromboembolic disorders. 2) A past history of deep vein thrombophlebitis or thromboembolic disorders. 3) Cerebral vascular or coronary artery disease. 4) Known or suspected carcinoma of the breast. 5) Known or suspected estrogen dependent neoplasia. 6) Undiagnosed abnormal genital bleeding. 7) Known or suspected pregnancy. 8) Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

Warnings:

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. Women who use oral contraceptives should be strongly advised not to smoke. Use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1) *Thromboembolic Disorders and Other Vascular Problems.* An increased risk of thromboembolic and thrombotic disease associated with use of oral contraceptives is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. In a study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater. An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported. Studies conducted in the United Kingdom found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction compared to nonusers who do not smoke. Oral contraceptive users who smoke have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. The amount of smoking is a very important factor. British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives; preparations containing 100 mcg. or more of estrogen were associated with a higher risk of thromboembolism than those containing 50–80 mcg. of estrogen. The relative risk of thromboembolic disease associated with progestin-only oral contraceptives has not been determined. Cases of thromboembolic disease have been reported in women using progestin-only products, and they should not be presumed to be risk-free. The overall excess mortality rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15–34, 5/100,000; ages 35–44, 33/100,000; ages 45–49, 140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. The highest risk was found in heavy cigarette smokers (15 or more cigarettes per day) who used oral contraceptives and were aged 40 or older. Women who smoke should be advised not to use oral contraceptives. The use of oral contraceptives in women over age 40 with other risk factors is not recommended. The mortality associated with all of the methods of birth control is low compared to the risk of childbirth, with the exception of oral contraceptive users who smoke and are over age 40. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and for myocardial infarction is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia, and especially by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. If feasible, oral contraceptives should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2) *Ocular Lesions.* There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue the medication if there is unexplained sudden or gradual, partial or complete loss of vision; sudden onset of proptosis or diplopia; papilledema; or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3) *Carcinoma.* Long term administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. In humans, one study investigated the first 21 cases of endometrial adenocarcinoma in women on oral contraceptives reported to a registry. Of those women without predisposing risk factors for this disease, nearly all occurred in women who had used a sequential oral contraceptive. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestin-only oral contraceptives. No increase in breast cancer in women taking oral contraceptives has been reported although one study reported an increased risk of breast cancer in subgroups of women treated using oral contraceptives with documented benign breast disease. There is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives. 4) *Hepatic Adenoma.* Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study reported a higher risk associated with oral contraceptive formulations with high hormonal potency. Although benign and rare, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short term as well as long term users of oral contraceptives. Two studies relate the risk with duration of contraceptive use, the risk being much greater after 4 or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5) *Use in Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring.* Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. A high percentage of such exposed women (30% to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Similar data are not available with the use of other estrogens but it cannot be presumed that they would not induce similar changes. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000 live births. In the past, female abortion. There is considerable evidence that estrogens are ineffective for these inc there is no evidence from well-controlled studies that progestins are effective for Increases in chromosomal aberrations have been reported in women who become p after ceasing oral contraceptive therapy. Embryos with these anomalies are virt spontaneously aborted. Whether there is an overall increase in spontaneous abortion o conceived soon after stopping oral contraceptives is unknown. It is recommended that f who has missed two consecutive periods, pregnancy should be ruled out before cc contraceptive regimen. If the patient has not adhered to the prescribed schedule, the pregnancy should be considered at the time of the first missed period and furthe contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is c patient should be apprised of the potential risks to the fetus and the advisability c continuation should be discussed in light of these risks. It is also recommended that discontinue oral contraceptives with the intent of becoming pregnant use an alter contraception for a period of time before attempting to conceive. Many clinicians recor months. Administration of progestin-only or progestin-estrogen combinations to induc bleeding should not be used as a test for pregnancy. 6) *Gall Bladder Disease.* Studies hav increased risk of surgically confirmed gallbladder disease appearing after one contraceptive use and doubling of the risk after 4 or 5 years of use. 7) *Carbohydr Metabolic Effects.* A decrease in glucose tolerance has been observed in a significant p patients on oral contraceptives. For this reason, prediabetic and diabetic patients shoul observed while receiving oral contraceptives. Increased serum levels of triglycerid phospholipids have been observed in oral contraceptive users. The clinical signific observation is unknown at this time. 8) *Elevated Blood Pressure.* An increase in blood p been reported in women receiving oral contraceptives. The prevalence of hyperten contraceptive users may be no higher than nonusers in the first year of oral contracep increases with longer exposure and in the fifth year of use is two and one-half to thr reported prevalence in the first year. Women who previously had hypertension during pre be more likely to develop elevation of blood pressure when given oral contraceptives. 9) The onset or exacerbation of migraine or development of headache of a new patte recurrent, persistent or severe, requires discontinuation of oral contraceptives and inv the cause. 10) *Bleeding Irregularities.* Breakthrough bleeding, spotting and amenorrhea reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as ir irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In u persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic m indicated to rule out pregnancy or malignancy. If pathology has been excluded, time c another formulation may solve the problem. Changing to an oral contraceptive with a high content, while potentially useful in minimizing menstrual irregularity, should be d necessary since this may increase the risk of thromboembolic disease. Women with a pa oligomenorrhea or secondary amenorrhea or young women without regular cycles tendency to remain anovulatory or to become amenorrheic after discontinuati contraceptives. Women with these preexisting problems should be advised of this pos encouraged to use other contraceptive methods. 11) *Ectopic Pregnancy.* Ectopic intrauterine pregnancy may occur in contraceptive failures. However, in oral contracept the ratio of ectopic to intrauterine pregnancies is higher than in women not using oral cor since the drugs are more effective in preventing intrauterine rather than ectopic preg higher ectopic-intrauterine ratio has been reported with both combination products and only oral contraceptives. 12) *Breast Feeding.* A small fraction of the hormonal age contraceptives has been identified in the milk of mothers receiving these drugs. The effect to the nursing infant cannot be determined at this time.

Precautions: 1) A complete medical and family history should be taken prior to the initia contraceptives. Examination should include special reference to blood pressure, breast and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a g oral contraceptives should not be prescribed for longer than 1 year without anoth examination being performed. 2) Under the influence of estrogen-progestogen pr preexisting uterine leiomyomata may increase in size. 3) Patients with a history depression should be carefully observed and the drug discontinued if depression recurs degree. 4) Because oral contraceptives may cause some degree of fluid retention, condi might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome renal insufficiency or asthma require careful observation. 5) Patients with a past history during pregnancy have an increased risk of recurrence of jaundice while receiving oral co therapy. If jaundice develops in any patient receiving such drugs, the medication discontinued. 6) Steroid hormones may be poorly metabolized in patients with imp function and should be administered with caution in such patients. 7) Oral contraceptive have disturbances in normal tryptophan metabolism which may result in a relative deficiency. The clinical significance of this is yet to be determined. 8) Serum folate lev depressed by oral contraceptive therapy. This may complicate subsequent pregnancy wit folate deficiency. 9) The pathologist should be advised of oral contraceptive therapy wh specimens are submitted. 10) Certain endocrine and liver function tests and blood compo be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TE to increased circulating total thyroid hormone, as measured by protein-bound iodine (I column, or T_3 by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting th TBG; free T_4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduce to metyrapone test. 11) The active yellow tablets and the inert green tablets in Ovcon-50 day regimens) and the inert green tablets in the 28 day regimen of Ovcon-35 contain FD&C 5 (tartrazine) which may cause allergic-type reactions (including bronchial asthma) susceptible individuals. Although the overall incidence of FD&C Yellow No. 5 (tartrazine) in the general population is low, it is frequently seen in patients who also ha hypersensitivity.

Information for the Patient: Detailed Patient Labeling has been prepared for use by the patie been made available for distribution by the pharmacist.

Drug Interactions: Reduced efficacy and increased incidence of breakthrough bleeding associated with concomitant use of rifampin. A similar association has been sugge barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Adverse Reactions: An increased risk of the following serious adverse reactions has been with the use of oral contraceptives (see **Warnings**): Thrombophlebitis, pulmonary coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, ga disease, congenital anomalies. There is evidence of an association between the following and the use of oral contraceptives, although additional confirmatory studies are needed: M thrombosis; benign hepatomas; neuro-ocular lesions, e.g., retinal thrombosis and opti The following adverse reactions have been reported in patients receiving oral contraceptiv believed to be drug related: Nausea and/or vomiting, usually the most common adverse occur in approximately 10% or less of patients during the first cycle (other reactions, as rule, are seen much less frequently or only occasionally); gastrointestinal symptoms abdominal cramps and bloating); breakthrough bleeding; spotting; change in menst dysmenorrhea; amenorrhea during and after treatment; temporary infertility after disconti treatment; edema; chloasma or melasma which may persist; breast changes (te enlargement, and secretion); change in weight (increase or decrease); change in cervical er cervical secretion; possible diminution in lactation when given immediately postpartum; c jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental de reduced tolerance to carbohydrates; vaginal candidiasis; changes in corneal curvature (ste intolerance to contact lenses. The following adverse reactions have been reported in us contraceptives, and the association has been neither confirmed nor refuted: Premen syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like s headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, nodosum, hemorrhagic eruption, vaginitis, porphyria.

Acute Overdosage: Serious ill effects have not been reported following acute ingestion of la of oral contraceptives by young children. Overdosage may cause nausea, and withdrawa may occur in females.

Mead Johnson LABORATORIES

C-V2
MJL

Increased incidence of spontaneous abortion in pregnancies complicated by maternal diabetes mellitus

Hamish W. Sutherland, M.B., Ch.B., and Colin W. Pritchard, Ph.D.
Aberdeen, Scotland

A retrospective sample of 164 pregnancies in the years 1956 to 1975 of 78 insulin-dependent diabetic women was examined to assess the extent of the risk of clinically recognizable spontaneous abortion relative to the expected risk in a comparable population. This study was done before the implementation of the policy to establish good control of maternal diabetes before conception was attempted. After control of data for pregnancy number, maternal age, and epoch, the risk of spontaneous abortion among the diabetic women was almost double the estimate of the expected risk ($p < 0.001$). (AM J OBSTET GYNECOL 1986;155:135-8.)

Key words: Insulin-dependent diabetes, clinically recognizable abortion, pathogenesis of abortion

In women with insulin-dependent diabetes mellitus the loss of viable fetuses has diminished coincident with the application of improved control of maternal glycemia throughout pregnancy, although it is not possible to separate the effects of improved control from the "secular trend"[1] and the evolution of modern obstetric care.

With the introduction of a similar approach of good control immediately before conception and during embryogenesis the fetal malformation rate also has been significantly reduced.[2]

Although there are some reports that suggest that the frequency of spontaneous abortion in women with insulin-dependent diabetes mellitus is similar to that observed in the general population,[3, 4] the difficulties in obtaining good control data have engendered circumspection.[5] Without reliable information about the population from which the relative risk of abortion for women with insulin-dependent diabetes mellitus could be quantified, the potential effects of prepregnancy care (including improved metabolic control for these women) on their abortion rate remains open to doubt.

The aim of this study is to evaluate the risk of clinically recognizable abortion in women with insulin-dependent diabetes mellitus compared to the risk in the population of the same area who were subject to the same secular trend in the period before the introduction of a program of planned pregnancy for women with diabetes mellitus.

From the Department of Obstetrics and Gynaecology, Medical School, University of Aberdeen, and the Medical Sociology Unit, Medical Research Council.
Supported by Hospital Endowment Research Trust Grants Nos. 471 and C369 from the Scottish Home and Health Department.
Received for publication April 15, 1986; accepted July 17, 1986.
Reprint requests: Dr. Hamish W. Sutherland, Department of Obstetrics and Gynaecology, University of Aberdeen, Aberdeen AB9 2ZE, Scotland.

Material and methods

Spontaneous abortion refers in the United Kingdom to pregnancy failures before the twenty-eighth completed week of gestation.

Before, during, and since the period of this study it has been basic policy in the Grampian area of Scotland to admit to the hospital all women with inevitable, missed, and incomplete abortion for evacuation of the uterus under anesthesia whether or not there is coincidental disease. The diagnosis of complete abortion is rarely made and usually only following retrospective appraisal of the evidence for pregnancy and histopathologic evidence from the curettage.

The analysis reported in this paper is based on a sample of the 78 Caucasian women with insulin-dependent diabetes mellitus for whom records were available from the Aberdeen Diabetic Clinic and who had known pregnancies while insulin dependent in the years 1956 to 1975. In general terms the mode of control of the diabetes was dietary regulation and twice daily long- and short-acting insulin administration with dose adjustment according to a sliding scale of insulin dosage dependent on frequent urine test results with Clinitest tablets (Ames Co., Elkhart, Indiana). After 1975 a more active policy of blood glucose self-monitoring and prepregnancy counseling has been pursued, and the findings presented here will form the basis for part of the assessment of that policy at a later date.

Over the 20-year period under consideration these women had a total of 164 pregnancies. This represents by far the majority of pregnancies to women with insulin-dependent diabetes mellitus in the area over that period because apart from the Aberdeen center, there

Table I. Pregnancy numbers of the sample of diabetic pregnancies by previous pregnancies included in the sample

Pregnancy number	*Previous pregnancies included*							*Total*
	0	*1*	*2*	*3*	*4*	*5*	*6*	
1	71	–	–	–	–	–	–	71
2	2	46	–	–	–	–	–	48
3	1	1	24	–	–	–	–	26
4	1	–	1	10	–	–	–	12
5	2	–	–	–	2	–	–	4
6	–	–	–	–	–	1	–	1
7	1	–	–	–	–	1	–	2
Total	78	47	25	10	2	2	–	164

Table II. Spontaneous abortions, births, and observed and expected incidence of spontaneous abortion for the sample of diabetic pregnancies

	Diabetic spontaneous abortion (n)	*Births (n)*	*Total (n)*	*Spontaneous abortion (%)*	*Estimate of expected incidence (%)*
All	28	136	164	17.07	8.84
Gestation >9 weeks	25	136	161	15.53	8.26

were no other facilities for diabetic pregnant women, public or private, in the Grampian area. Table I reports the pregnancy numbers of the sample according to the number of previous pregnancies included in the study. The sample includes the seventh pregnancy to one woman (which resulted in a live birth) who had had six previous live births. The sample includes three women who had two spontaneous abortions. In each of these cases it was the first and second pregnancies that ended in this way and the pregnancies continued beyond the ninth week of gestation.

Estimates for the "expected" incidence of spontaneous abortion were derived in the manner described below from data stored on the Aberdeen Maternal and Neonatal Data Bank,[6] which contains records of pregnancies occurring in Aberdeen dating back to 1950.

The question being asked is whether the incidence of spontaneous abortion among women with insulin-dependent diabetes mellitus differs significantly from that in the population from which they were drawn.

Given that the sample of pregnancies in women with insulin-dependent diabetes mellitus is heterogenous by age, pregnancy number, and year of pregnancy, equivalent population estimates of the incidence of spontaneous abortion are problematic. For this reason the expected incidence was derived in the following way: For each diabetic pregnancy the probability of spontaneous abortion was calculated for women of the same pregnancy number, age group (ages grouped as 16 to 19, 20 to 24, 25 to 29, 30 to 34, and 35+ years), and epoch (years grouped as 1956 to 1960, 1961 to 1965, 1966 to 1970, and 1971 to 1975) who had known pregnancies in Aberdeen. The average of these probabilities thus represents an overall probability of spontaneous abortion for a sample similar in pregnancy number, age group, and epoch composition to the sample of diabetic pregnancies.

Because of the possibility that spontaneous abortions in the early weeks of gestation were more likely to be reported among the diabetic group, this procedure was repeated excluding the three pregnancies to women with insulin-dependent diabetes mellitus which were lost before the tenth week of gestation and excluding spontaneous abortions before the tenth week of gestation in the estimation of the expected incidence.

Results

The numbers of spontaneous abortions, births, and the expected incidences are reported in Table II.

Using the χ^2 approximation to binomial probabilities to test the difference in the proportions, the probability of spontaneous abortion among the sample of pregnancies to insulin-dependent diabetic women is significantly greater ($p < 0.001$) than the expected estimate both for all the pregnancies ($\chi^2 = 12.79$) and for those continuing beyond the ninth week of gestation ($\chi^2 = 10.28$).

It is possible that the spontaneous abortions that occurred among the sample of diabetic women happened in pregnancy number by age group by epoch categories where the estimated probability of spontaneous abortion was greater than for the overall sample. The average estimated incidence of spontaneous abortions for those cells in which an abortion occurred in the diabetic

sample was 7.32%, or less than the overall estimate. Moreover, 18 of the 25 abortions (72.0%) occurring after the ninth week of pregnancy were in cells in which the incidence of spontaneous abortion was less than the overall estimate, which may be compared to 96 of the total of 161 (59.6%) in which the estimated incidence was less than the average estimated incidence.

To ensure that the finding of an increased risk of spontaneous abortion was not overestimated because of the independent effects of previous obstetric history, an analysis of the 71 first pregnancies in the sample was conducted. Eleven (15.49%) of those pregnancies ended in a spontaneous abortion. The risk was significantly greater ($\chi^2 = 4.32$; $p < 0.005$) than the population estimate of 8.07%.

The conclusion reached is that there was a significantly higher incidence of spontaneous abortion among the sample of women with insulin-dependent diabetes mellitus than would be expected on the basis of an estimate of the incidence in an equivalent sample of Aberdeen pregnancies.

Comment

Contrary to the conclusions of several previous published studies, analysis of these data show that there is a significantly increased risk of spontaneous abortion as a recognizable clinical event in women with insulin-dependent diabetes mellitus. It confirms the conclusions of Miodovnik et al.[7] in Cincinnati whose work in turn confirmed our preliminary findings.[8] In our population the increased risk for women with insulin-dependent diabetes mellitus is of the order of twofold in both first and later pregnancies, but this is far short of the overall spontaneous abortion rate of 30% in the Ohio diabetic women.[7]

The pathogenesis of abortion in women with insulin-dependent diabetes mellitus is unknown. Diabetic ketoacidosis is a rare event at such an early stage of pregnancy when hypoglycemia is much more commonly the form of metabolic derangement necessitating hospital admission.[9] Nonetheless, glycosylated hemoglobin levels are higher at the time of first booking at the prenatal clinic in the diabetic women who later abort than in those who deliver successfully.[10] Immunologic studies have characterized alterations in the fetomaternal relationship in diabetic pregnancy, but to our knowledge these have not been correlated with the degree of metabolic control in early pregnancy.[11] Maternal diabetes was the first condition in which early growth delay of the human embryo was detected by ultrasonic examination.[12] Initial growth delay is particularly evident in cases where the fetus is found later to be malformed,[13] and this has been shown to be associated with poor control of the maternal diabetes at the time of conception.[14] However, in women with insulin-dependent diabetes mellitus not all fetuses with initial growth delay are malformed or are aborted. The association of threatened abortion with initial growth delay measured by ultrasound in nondiabetic women has been reported, and the extent of the growth delay was found to relate to the risk of spontaneous abortion.[15] There is some indirect evidence that marked growth delay in the presence of maternal diabetes may cause very early abortion.[16] Unfortunately, data were not available concerning either the precise gestational age at abortion (since the proportion with missed abortion is unknown) or the incidence of fetal malformation in the aborted fetuses in this study. It was also not possible to assess the degree of maternal control of glycemia at the time preceding abortion, and no similar data exist from those in the sample who did not abort.

An advantage resulting from preconception management of women with insulin-dependent diabetes mellitus will be the facility for retrospective appraisal of blood glucose self-monitoring during the early weeks of the first trimester in diabetic women who do and do not abort. This may provide information useful in the assessment of the role of maternal glycemia in spontaneous and missed abortion in diabetic women. The effect of good maternal periconception control of diabetes on the risk of abortion requires evaluation.

The data in this study collected before such a policy was implemented in Aberdeen indicate that the clinical event of spontaneous abortion is approximately twice as likely to occur in insulin-dependent diabetic women as in a matched population control. This is based on a risk of clinically apparent spontaneous abortion in diabetic women of around 15% which is much less than the 26% and 30% found in Birmingham, England,[10] and Cincinnati,[7] respectively, where the potential for improvement in prognosis for the pregnancies of diabetic women is even more substantial and challenging.

REFERENCES

1. Pedersen J. The pregnant diabetic and her newborn. 2nd ed. Copenhagen: Munksgaard, 1977;198-210.
2. Fuhrmann K, Reiher H, Semmler K, Fischer F, Fisher M, Glockner E. Prevention of congenital malformations in infants of insulin dependent diabetic mothers. Diabetes Care 1983;6:316-8.
3. Kitzmiller J, Cloherty J, Younger MD, et al. Diabetic pregnancy and perinatal morbidity. Am J Obstet Gynecol 1978;131:560-80.
4. Crane JP, Wahl N. The role of maternal diabetes in repetitive spontaneous abortion. Fertil Steril 1981;36: 477-9.
5. Kalter H. Diabetes and spontaneous abortion. Am J Obstet Gynecol 1985;152:603.
6. Samphier M, Thomson B. The Aberdeen Maternity and Neonatal Data Bank. In: Mednick SA, Baert AE, eds. Prospective longitudinal research. London: Oxford University Press, 1981:61-5.
7. Miodovnik M, Lavin JP, Knowles HC, Holrogde J, Stys SJ. Spontaneous abortion among insulin dependent diabetic women. Am J Obstet Gynecol 1984;150:372-6.

8. Sutherland HW, Ford A, MacIntyre S, Pritchard CW. Psychosocial aspects of childbearing, fertility and abortion in diabetic mothers. In: Laron Z, Galatzer A, eds. Recent progress in medico social problems in juvenile diabetes. Basel: Karger, 1983:26.
9. Pedersen J. Course of diabetes during pregnancy. Acta Endocrinol (Copenh) 1952;9:342-64.
10. Wright AD, Nicholson HO, Pollock A, Taylor KG, Betts S. Spontaneous abortion and diabetes mellitus. Postgrad Med J 1983;59:295-8.
11. Galbraith RM, Sinha DP, Galbraith GMP, Faulk WP. Immunohistological studies of placentae from insulin dependent diabetic women. In: Sutherland HW, Stowers JM, eds. Carbohydrate metabolism in pregnancy and the newborn. Edinburgh: Churchill Livingstone, 1984;23-33.
12. Pedersen JF, Molsted-Pedersen L. Early growth retardation in diabetic pregnancy. Br Med J 1979;1:18-9.
13. Pedersen JF, Molsted-Pedersen L. Early growth delay predisposes the fetus in diabetic pregnancy to congenital malformation [Letter]. Lancet 1982;1:737.
14. Pedersen JF, Molsted-Pedersen L, Mortensen HB. Fetal growth delay and maternal haemoglobin A1C in early diabetic pregnancy. Obstet Gynecol 1984;64:351-2.
15. Mantoni M, Pedersen JF. Fetal growth delay in threatened abortion: an ultrasound study. Br J Obstet Gynecol 1982;89:525-7.
16. Pedersen JF, Molsted-Pedersen L. Early fetal growth delay detected by ultrasound marks increased risk of congenital malformation in diabetic pregnancy. Br Med J 1981; 283:269-71.

Immunohistochemical studies on collagen types in the uterine cervix in pregnant and nonpregnant states

Toshinari Minamoto, M.D., Katsuhiko Arai, D.V.M., Shun Hirakawa, M.D., and Yutaka Nagai, Ph.D.

Tokyo, Japan

The distribution of collagen types in pregnant and nonpregnant uterine cervices was examined by immunoperoxidase staining with the use of type-specific anticollagen antibodies. The nonpregnant cervix, composed of dense fibrous tissues, was diffusely stained with antibodies to type I and type III collagens. Type IV collagen was located only in the basement membrane region. In the cervix at term pregnancy, a marked decrease in fibrous connective tissue with increased proportions of smooth muscle fibers was characteristic, forming expanded spaces in between due to edema (clear spaces) with focal infiltrations of polymorphonuclear leukocytes. Type I and type III collagens were distributed only around smooth muscle fiber bundles with some fibroblasts attached. Type IV collagen was distributed in a linear fashion delineating individual smooth muscle fibers and vascular basement membranes. A possible role of infiltrated polymorphonuclear leukocytes in the dissociation of fibrous connective tissues of the cervix at term pregnancy is discussed. (Am J Obstet Gynecol 1987;156:138-44.)

Key words: Anticollagen antibody, collagen, immunohistochemistry, cervix in pregnancy

In contrast to the myometrium of the corpus, the uterine cervix in the nonpregnant woman is predominantly composed of dense connective tissue, the principal component of which is dense and tightly packed collagen fibers.[1] Smooth muscle fibers account for 10% to 15% of the normal cervical substance and are scattered sparsely and at random throughout the tissue. Histologic and biochemical studies confirmed these findings and showed that the content of smooth muscle fibers decreased from the upper cervix downward.[2]

During the period of term pregnancy to labor, the cervix reveals dramatic changes such as softening and ripening. The characteristics of the cervix are to permit it to carry out its vital but conflicting functions of acting as a barrier to retain the conceptus and opening at an appropriate time to permit the conceptus to be expelled from the uterus. Morphologic changes in softening and ripening of the cervix are characterized by dissociation and branching of collagen fibers into their fibrillar components and expansion of the interfibrillar spaces (clear spaces) due to edema.[1-3] Biochemical studies showed that the concentration of collagen in the cervix at term pregnancy was decreased, while glycosaminoglycans, especially hyaluronic acid, and water contents were increased.[4]

From the Department of Tissue Physiology, Medical Research Institute, Tokyo Medical and Dental University, and the Department of Obstetrics and Gynecology, School of Medicine, Toho University.
This work was supported in part by Grants-in-Aid for Special Project Research (Nos. 60114002 and 60127003) from the Ministry of Education, Science and Culture, Japan.
Received for publication April 15, 1986; revised June 30, 1986; accepted July 28, 1986.
Reprint requests: Yutaka Nagai, Ph.D., Department of Tissue Physiology, Medical Research Institute, Tokyo Medical and Dental University, 2-3-10, Kandasurugadai, Chiyoda-ku, Tokyo 101, Japan.

Table I. Clinical history of the patients examined

Case No.	Age (yr)	Duration of gestation	Cervical condition		Indication for cesarean section or hysterectomy
			Dilatation (cm)	Effacement (%)	
Nonpregnant					
1-9	38-49 (mean 45)				Hysterectomy for leiomyoma in all cases
Pregnant					
10	46	8 wk, 2 days			Hysterectomy for leiomyoma
11	39	9 wk			Hysterectomy for leiomyoma
12	25	38 wk, 6 days	1	30	Cephalopelvic disproportion, breech
13	38	39 wk, 1 day	0.5	10	Repeat cephalopelvic disproportion, breech
14	41	39 wk, 3 days	0.5	20	High-risk pregnancy
15	34	39 wk, 3 days			Hysterectomy for atony of uterus after spontaneous labor
16	32	39 wk, 6 days	0	10	Repeat cephalopelvic disproportion, fetal distress
17	30	40 wk	2	20	Cephalopelvic disproportion
18	26	42 wk, 3 days	5	50	Fetal distress

Our previous studies showed that the solubility of collagen, particularly type I collagen, in the cervix was significantly increased at term pregnancy.[5] In parallel, the activities of collagen-degrading enzymes such as collagenase and collagenolytic peptidase were also elevated with softening and ripening of the cervix.[6] Although the distribution and function of collagen types in various tissues have recently been elucidated,[7] little information is available as to those in the ripening process of the pregnant cervix at present.

Therefore we studied distributions of collagen types I, III, and IV in nonpregnant and pregnant cervical connective tissues by the immunoperoxidase staining method with type-specific anticollagen antibodies used to elucidate how collagen types are involved in dilatation and effacement of the cervix in term pregnancy.

Material and methods

Uterine cervical tissues from 18 women of child-bearing age were examined. Cervical tissues from nonpregnant women were obtained at the time of hysterectomy for leiomyomas, and uninvolved regions were used. Cervical tissues were also obtained from pregnant women at term at the time of cesarean section and from patients of 8 and 9 weeks' gestation with leiomyomas (Table I). These cervical tissues were fixed with 10% buffered formalin and embedded in paraffin for histochemical and immunoperoxidase staining.

Type-specific rabbit antibodies to type I collagen (from fetal calf skin) and rat antibodies to type III and type IV collagens (both from the human placenta) were purified by affinity chromatography.[8] Antibodies to types I, III, and IV collagens were shown to have reciprocal titers of 2^{11}, 2^{10}, and 2^{12}, respectively, by passive hemagglutination assays and showed no cross-reaction with other types of collagens.

Paraffin sections were routinely stained with hematoxylin and eosin, azan, and phosphotungstic acid–hematoxylin.

Immunoperoxidase staining was performed according to the method described by Sternberger et al.[9] Paraffin sections were pretreated with 0.01% protease (type VII, Sigma Chemical Co., St. Louis, Missouri) in Tris hydrochloride buffer, 0.05 mol/L, pH 7.6, at 37° C for 10 minutes and then incubated with antibodies to type I, type III, or type IV collagen, diluted to 1:10, 1:20, and 1:20, respectively with phosphate-buffered saline, 0.01 mol/L, pH 7.2. Swine immunoglobulin G to rabbit immunoglobulin G with rabbit peroxidase-antiperoxidase complex (Dakopatts, Glostrup, Denmark) and rabbit immunoglobulin G to rat immunoglobulin G (Cappel Laboratories, Cochranville, Pennsylvania) with rat peroxidase-antiperoxidase complex (Jackson Immuno-Research Laboratories, Inc., Avondale, Pennsylvania) were used in a dilution of 1:100 with phosphate-buffered saline. As a control, nonimmunized rabbit and rat immunoglobulin G was used instead of type-specific anticollagen antibodies.

Results

In the nonpregnant uterus, the cervix was mainly composed of dense fibrous connective tissues, among which some smooth muscle fibers were scattered sparsely. These two major fibrous components were intermingled with no particular alignment and were tightly packed with each other. Immunoperoxidase staining revealed that type I and type III collagens were diffusely distributed in the dense fibrous connective tissues with no remarkable differences shown in the distribution of these collagens (Fig. 1, *a* and *b*). Type IV collagen was observed in a fine linear fashion

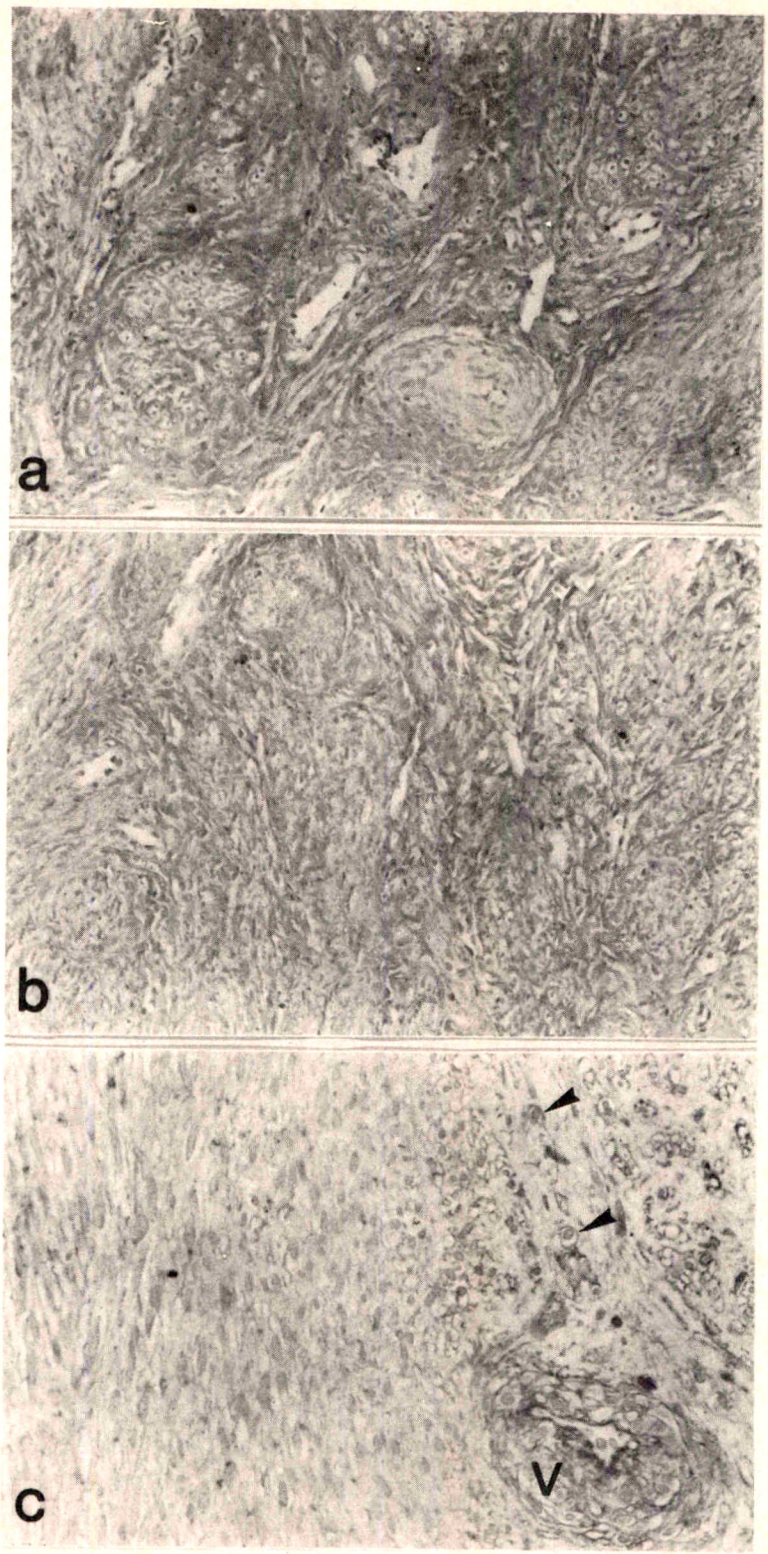


Fig. 1. Immunoperoxidase staining for type I, type III, and type IV collagen in the nonpregnant cervix. Type I *(a)* and type III *(b)* collagen was distributed diffusely in the dense fibrous tissue, while type IV collagen *(c)* delineated basement membranes. *V*, Blood vessel; *arrowheads*, immunoreactive components delineating smooth muscle fibers. (Original magnification ×200.)

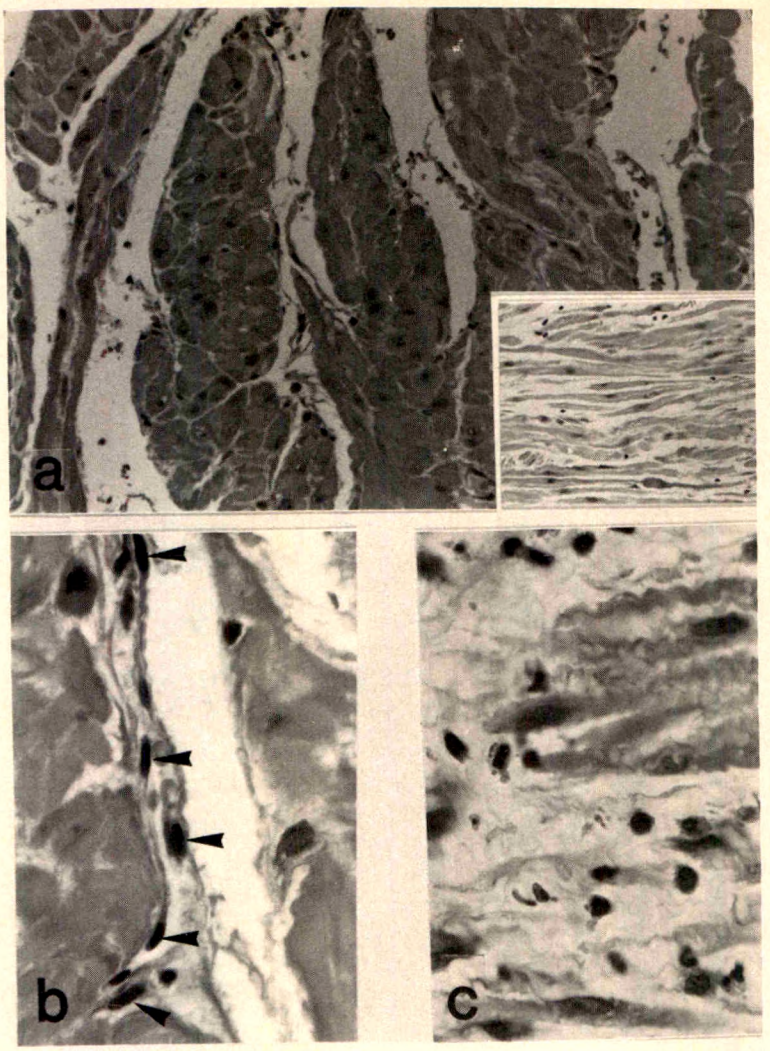


Fig. 2. The cervix of term pregnancy showing an increased proportion of smooth muscle fibers aligned in a particular direction and clear spaces in between *(a)*. Some smooth muscle fibers were locally dissociated (see *inset*) in the area where polymorphonuclear leukocytes were infiltrated (see also *c*). Some fibroblasts *(arrowheads)* were observed along with thin collagenous fibers around the smooth muscle fibers *(b)*. Polymorphonuclear leukocytes were focally infiltrated in the clear space *(c)*. (*a*, Including *inset*: Hematoxylin and eosin. Original magnification ×200. *b* and *c*: Hematoxylin and eosin. Original magnification ×400.)

at the blood vessels and along individual smooth muscle fibers showing the delineation of the basement membrane but was not distributed in the dense fibrous connective tissue (Fig. 1, *c*). Nonimmunized rabbit and rat immunoglobulin G showed no reaction with the tissue specimens examined (data not shown).

In term pregnancy, dissociated and hypertrophic smooth muscle fibers were aligned in particular directions (Fig. 2, *a*), and some smooth muscle fibers were locally dissociated (Fig. 2, *a*, *inset*) in the area where polymorphonuclear leukocytes were infiltrated (see also Fig. 2, *c*). The content of fibrous connective tissue components was markedly decreased and the remaining thin fibrous components were attached around smooth muscle fibers and bundles, where some fibroblasts were present (Fig. 2, *b*). The spaces between smooth muscle bundles or collagen fibers were expanded because of edema (clear space), and infiltrated polymorphonuclear leukocytes were focally observed in the clear space (Fig. 2, *c*). Immunoperoxidase staining with the use of anticollagen antibodies showed quite similar distributions of type I and type III collagen around smooth muscle bundles (Fig. 3, *a* and *b*), although type III collagen was also distributed within the bundles (Fig. 3, *b*). Type IV collagen delineated individual smooth muscle fibers and basement membranes

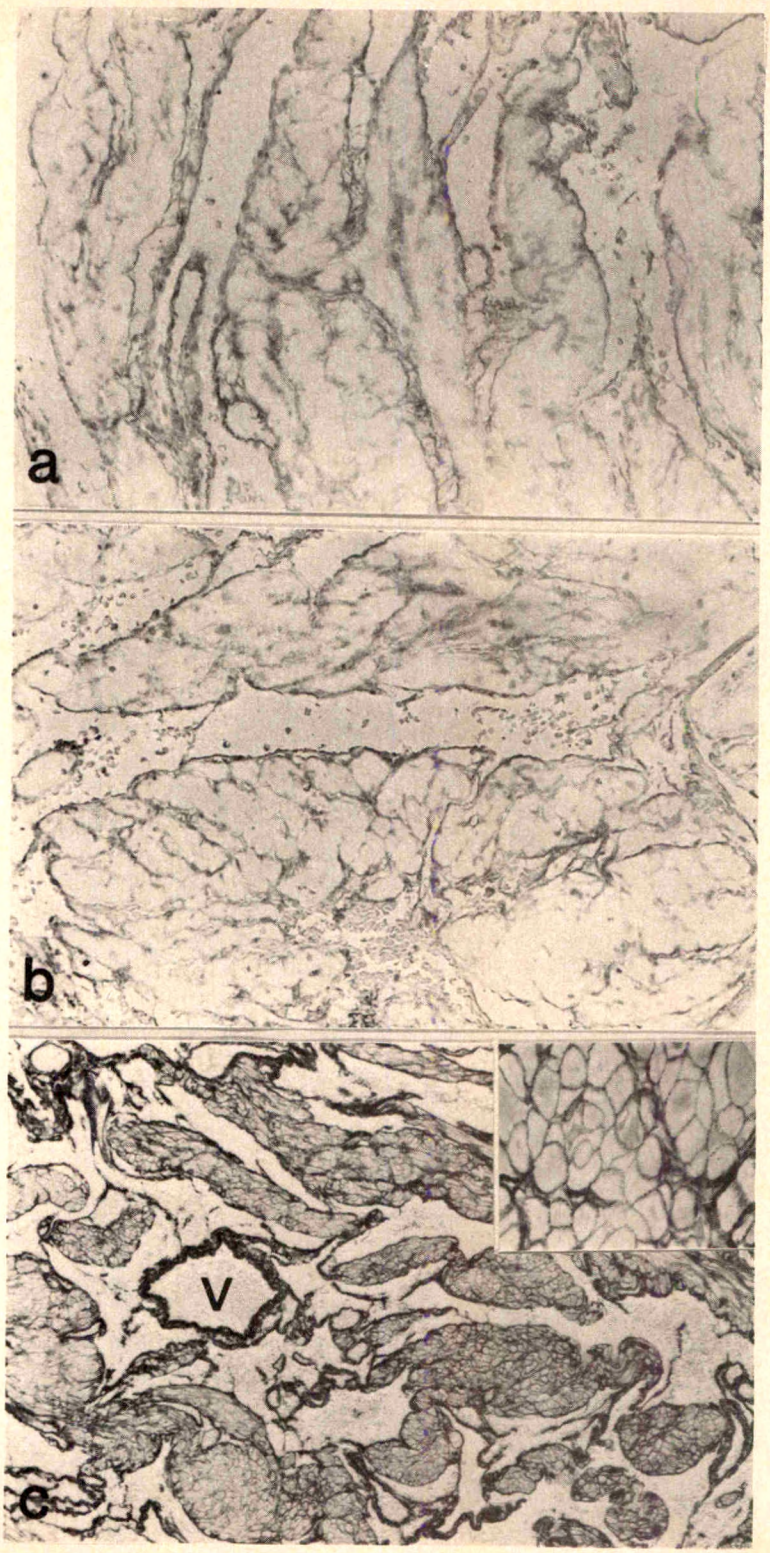


Fig. 3. Immunoperoxidase staining for type I *(a)* type III *(b)*, and type IV *(c)* collagen in the term pregnant cervix. The *inset* of *c* shows individual smooth muscle fibers outlined by type IV collagen. *V*, Blood vessel. (*a* and *b*: Original magnification ×200. *c*: Original magnification ×100, *Inset* of *c*: Original magnification ×250.)

of blood vessels (Fig. 3, *c*). The clear spaces and smooth muscle fiber itself did not react with any antibodies used, and nonimmunized rabbit and rat immunoglobulin G showed no reaction with the tissue specimens examined (data not shown).

In the cervical tissues of early gestation (8 and 9 weeks) compared with the nonpregnant cervix, little change was obsesrved in smooth muscle fibers although fibrous connective tissue regions became slightly sparse in pregnancy. The distribution of collagen types I, III, and IV resembled that of the nonpregnant cervix.

Comment

The uterine cervix plays active roles, namely, dilatation and effacement, during pregnancy and labor. Dilatation and effacement are not simply a result of mechanical forces but also depend on a ripening process involved in the cervix. The main process of ripening occurs in the last 3 to 4 weeks of pregnancy.[10] Ripening disorders may cause serious obstetric complications; that is, extensive ripening before term pregnancy may produce cervical incompetency, whereas delayed ripening or a rigid cervix at term may lead to prolonged labor or postterm delivery. For the resolution and prevention of these problems, elucidation of detailed mechanisms of the normal ripening process is indispensable.

Histologic examination of the cervix in term pregnancy showed that the cervical tissue became very loose, because of the decrease in and dissociation of collagen fibers and expansion of the clear space caused by edema. These observations agree with the findings previously described.[1, 3] The proportion of smooth muscle fibers that became hypertrophic increased in term pregnancy. A relatively higher population of smooth muscle fibers in our cases compared with those of previous reports[3] can be explained by the location of the specimens examined, that is, from the lower uterine segment adjacent to the obstetric internal os where cesarean section is generally performed. It is of interest that these smooth muscle fibers were aligned in particular directions (Fig. 2, *a*), which was in marked contrast to the fibers in the corresponding region, or between the anatomic and histologic internal ora, of the nonpregnant cervix (Fig. 1). The smooth muscle bundles likely play a role in the maintenance of the conceptus until delivery and prepare to extend in appropriate directions at delivery to prevent cervical laceration.

In the pregnant cervices examined, dense fibrous connective tissues were markedly dissociated, and thin collagen fibers were located exclusively around smooth muscle bundles with some fibroblasts. Biochemical analysis has indicated decreased concentration[4] and increased solubility[5] of collagen, especially type I collagen, at term pregnancy. The activities of collagen-degrading enzymes, including collagenolytic peptidase, were also elevated with ripening of the cervix.[6] Infiltration of polymorphonuclear leukocytes into the clear space seems to be closely related to collagenolysis,[3, 11-14] since our recent studies on the role of inflammatory cells in tissue (stroma) reactions have shown that, during infiltration of polymorphonuclear leukocytes from the bloodstream to the tissues, the inflammatory cells acquire a factor that stimulates connective tissue cells, including fibroblasts, to produce collagenase[11] and prostaglandin E_2[12] and to suppress collagen synthesis.[13] This sort of stromal reaction may also occur in the pregnant cervix in association with the collagen-degrading enzyme system directly derived from polymorphonuclear leukocytes,[3, 14] since recent studies have shown that intracervical application of prostaglandin E_2 induces the ripening process in the pregnant cervix.[15]

The functional characteristics of interstitial collagen are to provide tensile strength that is essential to hold tissues together as functional units, as well as to permit tissues to be pliable and/or extensible, and those of basement membrane (type IV) collagen are to serve as a stable backbone or supporting meshwork, as mechanical and ionic filters, and as a substratum for cell support and separation.[7] The distribution of collagen types I, III, and V and fibronectin in the corpus myometrium in normal and hypertensive pregnant patients was reported recently, with a description of the correlation between disruption of the uterine structure and the amount of urinary proteins excreted.[16] Another important observation is that prolonged dilatation time is closely related to increased concentrations of collagen in the lower part of the cervix.[4]

Immunohistochemical staining with the use of type-specific anticollagen antibodies revealed that type I and type III (interstitial) collagens were codistributed around smooth muscle bundles, but not in wide clear spaces, and that type IV (basement membrane) collagen delineated each smooth muscle fiber as well as the vascular basement membranes.

On the basis of these views, the distribution of collagen types in term pregnancy should serve the dual but conflicting functions of acting as a barrier to retain the conceptus and opening the cervix at the time of delivery. Further histologic and biochemical investigations on the ripening process in the pregnant cervix are currently in progress in our laboratory.

REFERENCES

1. Danforth DN. The morphology of the human cervix. Clin Obstet Gynecol 1983;26:7.
2. Rorie DK, Newton M. Histologic and chemical studies of the smooth muscle in the human cervix and uterus. Am J Obstet Gynecol 1967;99:466.

3. Junqueira LCU, Zugaib M, Montes GS, Toledo OMS, Krisztan RM, Shigihara KM. Morphologic and histochemical evidence for the occurrence of collagenolysis and for the role of neutrophilic polymorphonuclear leukocytes during cervical dilatation. Am J Obstet Gynecol 1980; 138:273.
4. Uldbjerg N, Ekman G, Malmström A, Olsson K, Ulmsten U. Ripening of the human uterine cervix related to changes in collagen, glycosaminoglycans, and collagenolytic activity. Am J Obstet Gynecol 1983;147:662.
5. Ito A, Kitamura K, Mori Y, Hirakawa S. The change in solubility of type I collagen in human uterine cervix in pregnancy at term. Biochem Med 1979;21:262.
6. Mori Y, Ito A, Hirakawa S, Kitamura K. Proteinases in the human and rabbit cervix. In: Ellwood DA, Anderson ABM, eds. The cervix in pregnancy and labour: clinical and biochemical investigations. Edinburgh: Churchill Livingstone, 1981:136.
7. von der Mark K. Localization of collagen types in tissues. Int Rev Connect Tissue Res 1981;9:265.
8. Konomi H, Hori H, Sano J, et al. Immunohistochemical localization of type I, II, III, and IV collagens in the lung. Acta Pathol Jpn 1981;31:601.
9. Sternberger LA, Hardy PH Jr, Cuculis JJ, Meyer HG. The unlabeleld antibody enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes. J Histochem Cytochem 1970;18:315.
10. Hendricks CH, Brenner WE, Kraus G. Normal cervical dilatation pattern in late pregnancy and labor. Am J Obstet Gynecol 1970;106:1065.
11. Nagai Y. The enzyme-mechanisms of tissue catabolism—a collagen-degrading enzyme system and degenerative diseases. In: Lobert L, Murata K, Nagai Y, eds. Degenerative diseases of connective tissue and aging. Tokyo: Kodansha, 1985:235.
12. Hashida R, Kobayashi S, Shirota H, et al. Stimulation of prostaglandin synthesis in cultured rat synovial cells by a factor derived from polymorphonuclear leukocytes. Prostaglandins 1984;27:697.
13. Ohsawa S, Hori H, Hata R, Nagai Y. Stimulation of the collagen metabolism of articular chondrocytes in culture by a factor derived from polymorphonuclear leukocytes. Biomed Res 1984;5:177.
14. Kobayashi S, Nagai Y. Human leucocyte neutral proteases, with special reference to collagen metabolism. J Biochem 1978;84:559.
15. Uldbjerg N, Ekaman G, Malmström A, Ulmsten U, Wingerup L. Biochemical changes in human cervical connective tissue after local application of prostaglandin E_2. Gynecol Obstet Invest 1983;15:291.
16. Pulkkinen MO, Lehto M, Jalkanen M, Näntö-Salonen K. Collagen types and fibronectin in the uterine muscle of normal and hypertensive pregnant patients. Am J Obstet Gynecol 1984;149:711.

Bound volumes available to subscribers

Bound volumes of the American Journal of Obstetrics and Gynecology are available to subscribers (only) for the 1987 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 156 (January-June) and Vol. 157 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the Journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular Journal subscription.

The planning of pregnancy among low-income women in central Harlem

Marie C. McCormick, M.D., Sc.D, J. Brooks-Gunn, Ph.D., Thomasine Shorter, M.S.W., Claudina Y. Wallace, M.D., M.P.H., John H. Holmes, B.S., and Margaret C. Heagarty, M.D.

Philadelphia, Pennsylvania, Princeton, New Jersey, and New York, New York

A planned pregnancy is considered desirable, in part because of the potential of a better pregnancy outcome. Since the improvement of pregnancy outcome is of particular relevance in low-income populations, we have compared the characteristics of women with planned and unplanned pregnancies in central Harlem with regard to those factors that might affect pregnancy planning such as sociodemographic factors, attitudes toward child-rearing, environmental stress, social support, and maternal mental health. Of the 416 women in the study, a minority (27%) reported their pregnancy as being planned. They differed from the remainder in being more likely to be married and/or living with a boyfriend or husband and to have been born outside New York City. The two groups did not differ in any other risk factor or in outcome in terms of birth weight and gestational age. The results provide little support for the lack of planning of pregnancy as an indicator of risk in a low-income population and suggest that improvement of perinatal outcome must involve more broadly based interventions that are not confined to the periconceptional period. (Am J Obstet Gynecol 1987;156:145-9.)

Key words: Planned pregnancy, low-income women, obstetric risk factors

A planned pregnancy is considered desirable for many reasons, and this desirability is reinforced by the observation of a statistical association between such pregnancies and better obstetric and perinatal outcomes.[1] However, the way in which planning for pregnancy may relate to pregnancy outcome remains to be established. One mechanism is the potential for prepregnancy screening for obstetric risk, although this relationship has received little empiric examination. Alternatively, women unable to plan their pregnancies may present higher obstetric risks for other reasons,[2, 3] for example, adolescent childbearing or reproductive problems that preclude effective contraception. Another factor may be increased ambivalence and anxiety in women with unintended or unwanted pregnancy, which may be associated with an increased risk for a variety of obstetric complications.[4, 5] Finally, absence of planning for pregnancy and increased obstetric risk may reflect other characteristics of the woman's environment which may affect pregnancy outcome such as physical and psychological stress and inadequate social support.[6-8]

The issue of pregnancy planning seems especially important to low-income populations in which lack of planning and perinatal morbidity are more common. Unfortunately, except for studies of low-income adolescent mothers, the factors associated with pregnancy planning in these populations have not been described. This paper describes the correlates of planning among a group of urban, low-income pregnant women.

Material and methods

Sample. The data for this study were collected as part of an evaluation of a program designed to provide enriched prenatal services in an urban low-income community, central Harlem. The sample consists of 599 women: 435 who enrolled for prenatal care at one of the four satellite clinics affiliated with Harlem Hospital and a sample of 164 women registering for prenatal care at the prenatal clinic within the Harlem Hospital Center during the period August 1982 and September 30, 1983. The sample represents 78% of all women eligible for the study. The remainder were eliminated as a result of spontaneous or elective abortions or because they did not continue care within the Harlem Hospital System.

Data collection. Each woman was asked to complete a self-administered questionnaire, generally during her

From the Division of General Pediatrics, Department of Pediatrics, Children's Hospital of Philadelphia, the Clinical Epidemiology Unit, Section of General Medicine, University of Pennsylvania School of Medicine, the Educational Testing Service, and the Department of Pediatrics, Harlem Hospital Medical Center.

This study was supported by grants from the Commonwealth Fund of New York City and by the Health and Hospitals Corporation of New York City.

Received for publication March 31, 1986; revised June 23, 1986; accepted July 11, 1986.

Reprint requests: M. C. McCormick, M.D., Sc.D., Pediatric Epidemiology and Health Services Research, 3440 Market St., Suite 420, Philadelphia, PA 19104-3306.

Table I. Sociodemographic characteristics of central Harlem women with planned (n = 113) and unplanned (n = 303) pregnancies

Sociodemographic factor	*Planned (%)*	*Unplanned (%)*	*Total (%)*
Age at start of antenatal care (yr)			
≤19	14.4	24.1	21.5
20-29	73.2	58.2	62.3
≥30	12.4	17.6	16.2
Educational attainment			
Less than high school graduation	43.1	46.8	45.8
High school graduate	35.3	35.2	35.2
Study beyond high school graduation	21.6	18.0	19.0
Born in New York City*			
No	46.7	34.8	38.1
Yes	53.3	65.2	61.9
Marital status*			
Married	31.2	16.4	20.4
Single	53.8	76.8	70.6
Other	15.0	6.8	9.0

*p-Value for χ^2 with appropriate degrees of freedom ($p < 0.05$).

first or second prenatal visit. Completed questionnaires were obtained from 76.5% (458) of the entire sample. Based on data abstracted from the obstetric record, it was found that those women who did not complete the questionnaire were more likely to be older, married, or classified as Hispanic or to have been born outside the United States. Respondents and nonrespondents did not differ with regard to the presence of historic or existing obstetric risk factors or the timing of their entry into prenatal care.

The research questionnaire consisted of questions in the following four areas:

1. Sociodemographic characteristics of the patients, which consisted of questions to ascertain age, educational level, birth place, duration of residence in New York City, marital status, membership in the maternal household, and family income.

2. Maternal attitudes toward the new child as determined by three questions concerning the mother's own assessment of her confidence about caring for the new child, her choice of names for the infant, and her previous child-rearing experiences. Included in these questions was a question as to whether the pregnancy was planned and a question as to whether the woman was surprised at being pregnant.

3. Maternal health behavior which involved questions about maternal smoking[9, 10] and drinking behavior derived from standard questionnaires.

4. Maternal exposure to stressful events, social support, and mental health. The first was assessed with use of a modification of the Dohrenwend's PERI scale,[11] which ascertained the presence of 17 stressful events in the year before the start of antenatal care. Maternal social support was ascertained by asking the women what they would do in each of six hypothetical situations: two in which they needed to borrow a modest amount of food or money, two in which they required assistance during an illness of varying durations, and two in which they needed advice or emotional support. The items were scored to indicate the number of situations in which the women could name an individual (by name and relationship to them) to whom they felt they could turn for help.[12] Mental health status was estimated by the 22-item version of the General Health Questionnaire,[13] which assesses general well-being, depression, anxiety, and somatic complaints. Scores above 5, with use of the dichotomous scoring system, have been shown to indicate clinically important mental distress.

In addition to the above items, previous reproductive history, birth weight, and gestational age were abstracted from the medical record.

All methods were reviewed and approved annually by the Institutional Review Board of the University of Pennsylvania. Differences in the distribution of categorical variables between those with planned pregnancies and those without were tested with use of χ^2, with Yates correction. All analyses were done with use of the Biomedical Data Package.[14]

Results

Of the 458 women who completed the questionnaire, 416 (91%) answered the question, "Did you plan this pregnancy?"; most of these women (303, or 73%) described their pregnancy as unplanned. Reinforcing the notion that most pregnancies were unplanned was the fact that most of those with unplanned pregnancies (198, or 65%) also reported themselves as surprised by the pregnancy.

Sociodemographic characteristics. As seen in Table I, many (46%) of these women had not completed high school and most (71%) were single. A smaller but substantial proportion (22%) were adolescent. Most were born in New York City. Married women and those born outside New York City were statistically more likely to report a planned pregnancy. The differences in maternal ages between those with planned and those with unplanned pregnancies approached a level of statistical significance ($p = 0.06$), with younger women being somewhat more likely to report their pregnancy as unplanned.

Maternal attitudes toward the infant. Although for the majority (58%) of women this pregnancy represented the birth of their first child, most (86%) expressed confidence in their child-caring ability; 53% had already selected a name for the anticipated infant.

Table II. Health behavior patterns of central Harlem women with planned (n = 113) and unplanned (n = 303) pregnancies

Behavior pattern	*Planned (%)*	*Unplanned (%)*	*Total (%)*
Cigarette smoking			
Never	44.6	42.6	43.2
Before pregnancy/not now	15.2	15.8	15.6
Less than before pregnancy	23.2	26.2	25.4
Same or more	17.0	15.4	15.9
Alcohol use			
Never	39.6	39.7	39.7
Before pregnancy	56.6	49.2	51.1
Now	3.8	11.2	9.2
Trimester of start of prenatal care			
First	43.2	36.2	38.0
Second	48.6	48.4	48.5
Third	8.1	15.4	13.6

None of these variables discriminated between those who planned and those who did not plan the pregnancy. However, those with planned pregnancies were marginally more likely to express confidence about their child-caring ability (91% versus 84%, $\chi^2 = 2.89$, $p = 0.09$).

Maternal health behaviors. More than half (57%) of these women reported that they smoked, but 25% reported that they were smoking less; 16% reported that they had stopped smoking completely during their pregnancy (Table II). Although most women reported alcohol use, few (9%) reported continued use during the pregnancy. Most (62%) enrolled in prenatal care after the first trimester of pregnancy.

Neither the use of tobacco or alcohol nor the timing into prenatal care discriminated between those who did and those who did not plan their pregnancies. Women who planned were marginally more likely to stop drinking during pregnancy than those who did not.

Maternal stress, social support, and mental health. Most women (71%) had experienced at least one major stressful event (excluding pregnancy) in the year before conception (Table III). Moreover, these mothers' levels of social support were low; few (29%) lived with a husband or boyfriend, and the majority (56%) were unable to name anyone who could help them in four or more of the presented hypothetical situations. In view of these findings, it is not surprising that a substantial proportion of these women (30%) scored in the distressed range of the General Health Questionnaire. Women who reported living with a husband or boyfriend were statistically more likely to have planned their pregnancies.

Previous reproductive history and pregnancy outcome. Among these women, almost 10% had had a previous low birth weight infant, and 14% had experienced at least one spontaneous abortion, with 22% having had an elective abortion (Table IV). None of these factors were associated with the planning of pregnancy. Of the 361 with obstetric outcome data (87% of those who delivered at Harlem Hospital), 10% weighed <2500 gm and 15% were preterm (<38 weeks' gestation). The outcome of pregnancy in terms of the infant's gestational age or birth weight did not differ with the presence or absence of maternal pregnancy planning.

Table III. Stressful events, social support, and mental health among central Harlem women with planned (n = 113) and unplanned (n = 303) pregnancies

Psychological/social factor	*Planned (%)*	*Unplanned (%)*	*Total (%)*
No. of stressful events in past year			
0	30.1	28.3	28.8
1	24.8	33.6	31.2
2	18.6	19.7	19.4
3+	26.5	18.4	20.6
Lives with husband/boyfriend*			
No	61.9	74.0	70.8
Yes	38.1	26.0	29.2
No. of situations with someone named as helper			
0	12.4	16.5	15.4
1-3	39.9	39.9	39.9
4-6	47.7	43.6	44.7
General Health Questionnaire			
≤5	74.3	67.8	69.5
>5	25.7	32.2	30.5

*p-Value for χ^2 with appropriate degrees of freedom ($p < 0.05$).

Table IV. Previous reproductive experience among central Harlem women with planned (n = 113) and unplanned (n = 303) pregnancies

Reproductive experience	*Planned (%)*	*Unplanned (%)*	*Total (%)*
Previous elective abortion			
No	77.0	78.0	77.7
Yes	23.0	22.0	22.3
Previous spontaneous abortion			
No	84.1	86.8	86.1
Yes	15.9	13.2	13.9
Previous low birth weight infant			
No	90.3	90.5	90.4
Yes	9.7	9.5	9.6

Comment

Variations in terminology make cross-study comparisons of intendedness of pregnancy difficult. The available national data[2] are developed from questions about whether the pregnancy is "wanted" and whether it is

wanted at the time of its occurrence. With use of these questions, the percentage of "unwanted" pregnancy is declining and is currently at a level of about 10%. However, of those pregnancies defined as wanted, more than one quarter (28%) are defined by the respondents as mistimed. Thus more than a third of women consider their pregnancies unintended at the time of conception. In populations similar to the present study (low income, urban, never married), the proportions of unwanted or mistimed pregnancies are substantially higher: 30% unwanted and 41% mistimed, which suggests that as many as 59% of pregnancies in these women are not intended.

In this study the percentage of "unplanned" pregnancies is somewhat higher (73%). However, we deliberately inquired about planning rather than wanting because of a concern that social desirability might lead to underreporting of unwantedness during an interview. Thus women who do not plan but want the pregnancy when it occurs may be one source of discrepancy and may reflect an underestimate of the wantedness of pregnancy in urban low-income populations. On the other hand, the national data, may overestimate wantedness, because the number of women who report a pregnancy as wanted increases with time as those who did not intend to become pregnant begin to accept and anticipate the birth of a child. Regardless of the precision of either data set, these data indicate that despite the availability of effective contraception, substantial numbers of pregnancies of urban low-income women in this country are unplanned at their conception.

Although the high proportion of unintended pregnancies was not unexpected, the failure of many of the usual socioeconomic and life-style variables to discriminate among the groups who planned and those who did not plan their pregnancies was not expected. These data suggest that urban low-income women who plan their pregnancies are somewhat more likely to be older, to live with a husband or boyfriend, to have been born outside the city of their current residence, and to be more confident about their ability to cope with the new baby. In addition, the concept of "covert" pregnancy planning is not supported by this study; no differences were found among the women who did not plan but were not surprised by the fact of pregnancy and those who did not plan but did report surprise by the event.

These results do not support the conventional image of the urban low-income mother with an unintended or unplanned pregnancy as one whose high levels of stress and lack of a social support system or mental distress hamper her ability to use reliable contraceptive methods. However, women who planned their pregnancy are significantly more likely to have the social stability involved in a relationship with a man, at least to the extent of living with a husband or boyfriend. These data support the findings of other investigations of low-income women and their decisions about abortion or continuation of pregnancy that the characteristics of the woman's relationship with her male partner are the most predictive factor of a decision to continue her pregnancy.[15] Both this study and previous work argue for the proposition that reproductive decisions of low-income women are made in the context of their specific situations and relationships and cannot be predicted by relatively static characteristics of the woman.

The absence of significant differences in obstetric outcome among women who did and those who did not plan their pregnancies reflects similarities in the presence of factors known to influence pregnancy outcome (tobacco use, timing of prenatal care, etc.). This lack of difference between those with planned and unplanned pregnancies indicates a missed opportunity, since even those with planned pregnancies are not able or encouraged to initiate their prenatal care early or to alter behaviors known to affect obstetric outcome adversely.

Our results differ from some investigations that have noted an association between unwanted pregnancy and poor obstetric outcome.[16] The discrepancy between these findings and the present study may reflect differences in the populations studied and in the influence of social class, or more specifically poverty, as mediators of the effects.

The limitations of these results must be noted. First, the study population consists of low-income women who seek care from clinics affiliated with a public general hospital; it does not include those who seek care outside this system or those who receive no prenatal care before delivery. Second, not all eligible women completed the baseline questionnaire. While comparisons of respondents with nonrespondents revealed no differences in incidence of prenatal problems or obstetric outcome, nonrespondents tended to be older, to be categorized as Hispanic, and to enroll in the earlier stages of the program. These differences can in part be explained by early logistic problems in program implementation and an initial lack of a Spanish version of the questionnaire.

Despite these limitations, these findings have important implications for the development of programs of reproductive services for urban low-income populations. If high levels of unplanned or unintended pregnancies are continuing among urban low-income women and if no obvious maternal characteristics permit discrimination between those who will from those who will not plan, two separate strategies for intervention seem necessary.

First, preconceptional care programs as recommended by the recent Institute of Medicine report[1] must involve approaches that do not require previous

knowledge of the timing of pregnancy and must be directed to the entire population of urban low-income women. In view of the apparent situational nature of the reproductive decisions of these women, multiple strategies for counseling low-income women about the adverse risks of pregnancy must be undertaken. The entire health and educational system with responsibility for these women, not just the obstetric and family planning community, must become involved in a systematic and consistent pattern of counseling about adverse health behaviors, smoking, need for prenatal care, contraceptive use, and the need for appropriate spacing of pregnancies. Considerable efforts must be directed toward training other health professionals, such as pediatricians and general physicians, in these matters so important to the health status of low-income pregnant women and their children.

Second, these results reinforce the importance of aggressive management of medical problems and adverse health behaviors as early in pregnancy as possible. This study presents a picture of a group of women, regardless of whether they have planned their pregnancies, who are under high levels of environmental stress and emotional distress and many of whom continue to smoke or to use excessive alcohol. Although traditional medical services have done much to improve the morbidity of pregnancy, these services can be anticipated to make no more than a marginal contribution to the health status of this group of pregnant women. Additional supportive services specifically designed to address these problems must be added to prenatal care services if additional improvement is to be expected.

We wish to acknowledge the insights and critical contributions of Vanessa Smith, Anna Soto, Brenda Williams, Sonya Branch, Agnes Cunningham, Arnecia Morgan, and Barbara Price and the helpful comments from consultants and staff of the Commonwealth Fund, as well as the assistance of Dr. David Cornfeld and Merceda Lafferty in preparation of the manuscript.

REFERENCES

1. Preventing low birth weight. Washington DC: National Academy of Sciences Press, 1985.
2. Pratt WF, Horn MC. Wanted and unwanted childbearing: United States 1973-82—advanced data. Hyattsville, MD: National Center for Health Statistics, 1985; DHHS publication no. (PHS)85-1250.
3. Klerman LV, Jekel JF. Unwanted pregnancy. In Bracken MD, ed. Perinatal epidemiology. New York: Oxford University Press, 1984.
4. Laukaran VH, Vandenberg BJ. The relationship of maternal attitude to pregnancy outcomes and obstetric complications. Am J Obstet Gynecol 1980;136:374-9.
5. Standley K, Soule B, Copans SA. Dimensions of prenatal anxiety and their influence on pregnancy outcome. Am J Obstet Gynecol 1979;135:22-6.
6. Norbeck JS, Tilden VP. Life stress, social support and emotional disequilibrium in complications of pregnancy: A prospective, multivariate study. J Health Soc Behav 1983;24:30-46.
7. Nuckolls KB, Cassel J, Kaplan BH. Psychosocial assets, life crisis and the prognosis of pregnancy. Am J Epidemiol 1972;95:431-41.
8. Newton RW, Webster PH, Binu PS, Maskrey N, Phillips AB. Psychosocial stress in pregnancy and its relation to the onset of premature labor. Br Med J 1979;2:411-3.
9. Landesman-Dwyer S, Keller LS, Streissguth AP. Naturalistic observations of newborns: effects of maternal alcohol intake. Alcoholism Clin Exp Res 1978;2:171-7.
10. Calahan D, Cisin I, Crossley H. American drinking practices: a national study of drinking behavior and attitudes. New Brunswick: Rutgers University Press, 1969.
11. Dohrenwend BS, Krasnoff L, Askenacy AR, Dohrenwend BP. Exemplification of a method for scaling life events: the PERI life events scale. J Health Soc Behav 1978; 19:205-29.
12. Cohen JB, Lazarus RS. Social supports questionnaire. Berkeley, California: University of California, 1977.
13. Goldberg DP. The detection of psychiatric illness by questionnaire. Maudsley Monograph No. 21. London: Oxford University Press, 1972.
14. Dixon WJ, ed. BMDP statistical software 1981. Berkeley, California: University of California Press, 1981.
15. Bracken MB, Klerman LV, Bracken M. Abortion, adoption, or motherhood: an empirical study of decision-making during pregnancy. Am J Obstet Gynecol 1978; 130:251-62.
16. Binkin N, Mhango C, Cates W, et al. Women refused second-trimester abortions: correlates of pregnancy outcome. Am J Obstet Gynecol 1983;145:279-84.

Ultrasonically evident fetal nuchal skin thickening: Is it specific for Down syndrome?

Ants Toi, M.D., Gary F. Simpson, M.D.,* and Roy A. Filly, M.D.

Hamilton, Ontario, Canada, and San Francisco, California

Fetal nuchal thickening in the second trimester is suggested as an ultrasonographic sign strongly suggesting the presence of Down syndrome. To better understand this potentially valuable observation, we explored aspects of the finding not considered in previous reports. The published data were reanalyzed to estimate statistically the presence of the sign in a low-risk obstetric population. Second, films of chromosomally normal fetuses were reviewed to confirm the normal range of measurement. Finally, autopsy reports of aborted Down syndrome fetuses were reviewed. Our results confirm that true nuchal thickening is present in some second-trimester Down syndrome fetuses. Unfortunately, a positive sign occurs in a significant proportion of chromosomally normal fetuses. Also our calculations predict a high incidence of false positive results in a low-risk obstetric population. Consequently we urge caution in the interpretation of these findings until prospective characterization of these observations is available. (Am J Obstet Gynecol 1987;156:150-3.)

Key words: Down syndrome, ultrasonics, fetus

Down syndrome has been diagnosed by pediatricians for many years by its phenotypical external features.[1,2] Previous authors emphasize that multiple characteristics need to be present before a confident diagnosis can be suggested. They also stated that any of the multiple findings in Down syndrome infants may be found in chromosomally normal children.

Recently two reports described an ultrasonographically visible sign that was highly specific for predicting the presence of Down syndrome in the second trimester of pregnancy. The sign is an abnormal skin thickness of greater than 5 mm behind the neck.[3,4] In the reported combined patient series this finding was present in four of 11 (36%) Down syndrome fetuses and in only one chromosomally normal fetus. The authors further suggested that if the sign is present, then "it appears to be highly specific and should lead to counseling the parents regarding its implications and their options." The serious clinical implications of these suggestions prompted us to analyze our own material to better understand this potentially valuable ultrasonographic observation.

From the Department of Radiology, McMaster University Medical Center, and the Departments of Obstetrics, Gynecology and Reproductive Sciences and Radiology, University of California, San Francisco.

Received for publication February 19, 1986; revised July 22, 1986; accepted August 14, 1986.

Reprint requests: A. Toi, M.D., Department of Radiology, Toronto General Hospital, 200 Elizabeth St., Toronto, Ontario, Canada M5G 2C4.

**Fellow in Reproductive Genetics and Ultrasound.*

Table I. Calculated changes in test performance as incidence of Down syndrome changes in the population

	Test population	*All pregnancies*	*Pregnancies in women under age 30*
Incidence	1:155	1:660	1:1500
Sensitivity (%)	36.4	36.4	36.4
Specificity (%)	99.9	99.9	99.9
Positive predictive value (%)	80.0	48.3	19.6
Negative predictive value (%)	99.6	99.9	99.9

With the standard 2 × 2 table, if we know the incidence, sensitivity, specificity, we can calculate the new contents of each cell as the incidence changes. This then allows us to estimate the new positive and negative predictive values in test populations that have differing incidences of disease.[6]

Methods

We studied the following questions: (1) What would be the expected performance of this test in the general obstetric population that is at low risk for Down syndrome? (2) Is this sign visible in chromosomally normal fetuses? (3) How frequently is nuchal thickening evident in Down syndrome fetuses aborted in the second trimester?

Test performance in low-risk population. The published data were analyzed with a standard 2 × 2 decision matrix that helps to determine the usefulness of a diagnostic test.[5] These results were then used to estimate the expected performance of this test in the normal low-risk obstetric population.

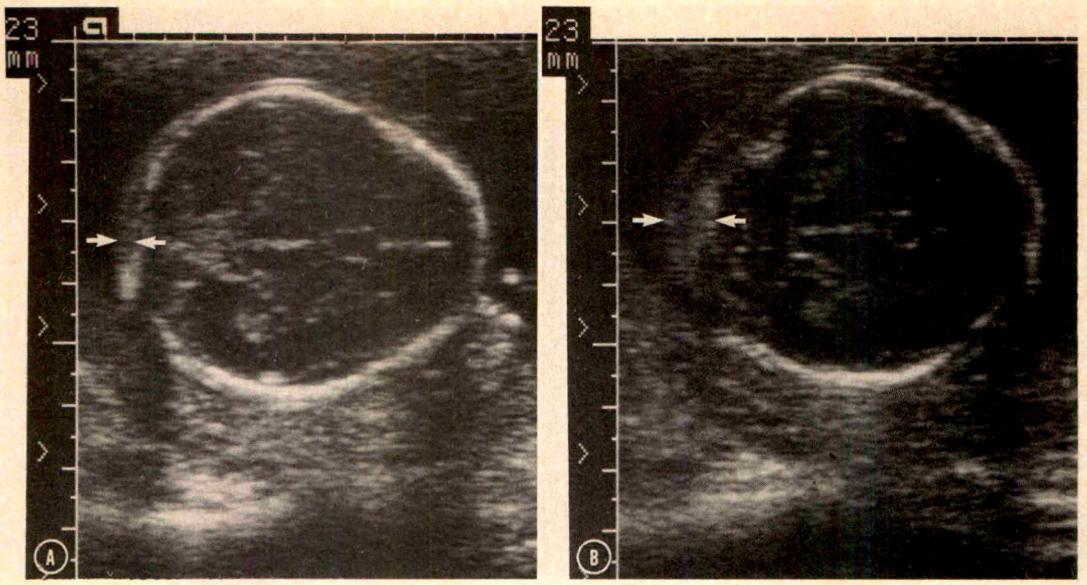


Fig. 1. *A*, Ultrasonogram of normal fetal head at 21 weeks' gestation showing normal skin thickness *(arrows)* behind the occiput. Here the plane of section is not steeply angled to the baseline. It is cephalad to the cerebellum and includes the occipital lobes and ambient cistern. *B*, Ultrasonogram of same fetus showing nuchal thickening of 8 mm measured as indicated by the *arrows*. Note that the plane of section in this image is more steeply angled to the baseline since the cerebellum and cisterna magna are included in the section.

Is the sign visible in chromosomally normal fetuses? We reviewed the ultrasonograms of 28 pregnancies undergoing detailed ultrasonic assessment for elevated amniotic fluid α-fetoprotein levels. This group was selected since all of these fetuses had been proved to be chromosomally normal and were in the same gestational age range as those of the previous studies. The gestational ages ranged from 18.5 to 25.5 menstrual weeks. Nuchal tissue thickness was measured on transaxial images of the fetal head that were suitable for biparietal diameter measurement. The planes of section included the thalamus, third ventricle, and occipital tissues. The distance from the external surface of the occipital bone to the external surface of the skin was measured. If the scan angle was such that the posterior part of the plane of section was caudal to the ossified part of the occipital bone, then the thickness of tissue between the cisterna magna and the external surface of the skin was taken. In each instance, the maximal value was recorded.

In an additional seven fetuses with normal chromosomes verified by amniocentesis, we deliberately set out to see if we could create a picture showing nuchal thickening by varying the angle of the plane of section while maintaining a transaxial orientation that always included the third ventricle and thalamus.

How often does nuchal thickening occur in Down syndrome fetuses in the second trimester? The autopsy reports of fetuses aborted with amniocentesis-verified Down syndrome at the University of California, San Francisco, were reviewed with attention to external soft tissue changes, especially in the region of the neck. Thirteen reports were available. Menstrual age ranged from 18 to 23 weeks. Since no anthropomorphic measurement criterion is available for nuchal skin thickness, we relied on the experience of the examining pathologist to visually differentiate thickened from normal neck tissues.

Results

Test performance in the low-risk population. In the combined published reports, 1704 patients at 15 to 20 weeks' gestation were assessed for nuchal thickening at the time of genetic amniocentesis. Eleven fetuses had Down syndrome, for an incidence of 11/1704, or one case per 155 patients. The positive finding of isolated nuchal thickening was present in four and absent in seven Down syndrome fetuses. One of the seven, however, had a cardiac anomaly and hydrops, a combination that also suggests Down syndrome. In only one instance was the finding present in a chromosomally normal fetus.

This allows the following calculations: incidence = 11/1704 (1:155); sensitivity = 4/11 (36%); specificity = 1692/1693 (99.9%); positive predictive value = 4/5 (80%); negative predictive value = 1692/1699 (99.6%).

This test population had an unusually high incidence

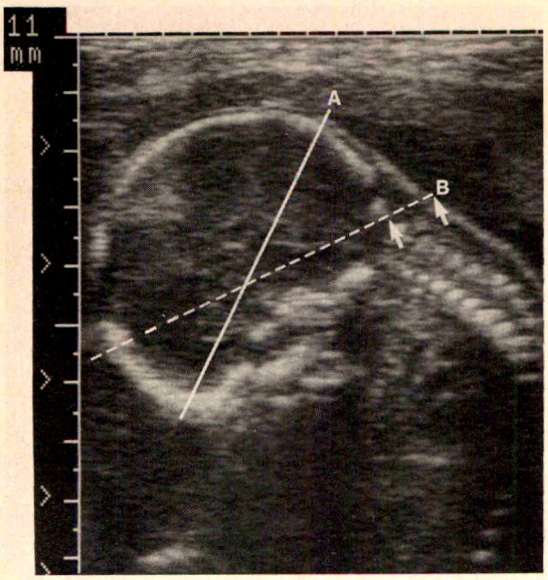


Fig. 2. Dorsal midsagittal ultrasonogram of a chromosomally normal fetus showing the normal nuchal soft tissues. A transverse head image obtained along plane *A* would give an image as in Fig. 1, *A*, with "thin" occipital tissues. However, a steeply angled image along plane *B* would appear as in Fig. 1, *B*, and show spurious thickening of nuchal tissues *(arrows)* as a sectioning artifact. The transverse ultrasonogram along plane *B* would show the lower cerebellum and cisterna magna.

of Down syndrome (1:155). Under these conditions a positive test indicates that four of five (80%) fetuses will have Down syndrome.

If we know the incidence of the disease in another population, for instance, the normal low-risk obstetric population, then we can estimate the expected performance of the test in that instance.[6] The published incidence of Down syndrome in mothers between 15 and 29 years of age is 1:1500.[7]

The calculations give the following results (Table I): sensitivity = 36%; specificity = 99.9%; positive predictive value = 20%; negative predictive value = 99.9%.

Note that the sensitivity and specificity have not changed but the clinically important positive predictive value has decreased to 20%.

Is the sign visible in chromosomally normal fetuses? In our study, nuchal thickening beyond 5 mm was present in six of 28 (21%) chromosomally normal fetuses examined for increased α-fetoprotein values in the amniotic fluid and ranged from 6 to 12 mm. None of these fetuses had hydrops, generalized skin thickening, or fluid collections in serous cavities. Only one had an anomaly, that being a mild dilation of cerebral ventricles without any other detectable abnormality. This anomalous fetus did not have evident nuchal thickening. When nuchal thickening was found, it was always present on a section taken for biparietal diameter measurement, which included the thalamus and lower cerebellum and cisterna magna.

In the group of seven chromosomally normal fetuses in whom we deliberately set out to see if we could create a falsely thickened neck, we found that as the image plane became more steeply angled caudally and intersected lower levels of the cerebellum and occiput, we were always able to show skin thickness >5 mm (Figs. 1, *A* and *B*, and 2).

How often does nuchal thickening occur in Down syndrome fetuses in the second trimester? There were 13 autopsy reports available. The neck was specifically stated to be normal in six abortuses. An additional three abortuses were noted to be externally normal, but the neck was not specifically mentioned. A further fetus had severe nonimmune hydrops. Another was macerated after spontaneous death in utero. Neck thickening was evident in two fetuses. This gives an incidence of two fetuses with isolated nuchal thickening in a total of 11 (18%) if the hydropic and macerated fetuses are excluded.

Unfortunately we cannot comment on the ultrasonographic frequency of occurrence of nuchal thickening at the University of California, San Francisco, Hospital since patients undergoing amniocentesis because of late maternal age have only a limited-guidance scan without detailed fetal assessment. Images were available for only a few patients in whom a major finding, such as severe hydrops, was evident at the time of amniocentesis.

Comment

Most Down syndrome infants are born to women under age 35 who have insufficient risk to warrant genetic amniocentesis. In addition, only about a third of women over age 35 undergo amniocentesis.[4] Since many pregnant women undergo ultrasonographic examination, it would be very useful to have an easily detectable, reliable ultrasonographic finding, such as nuchal thickening, to identify affected fetuses early enough in the second trimester so that there is time for possible intervention.

A review of the literature reveals very little information about Down syndrome appearances in the second trimester. This is in contrast to the large body of information describing Down syndrome in neonates at term. Hall[1] evaluated 48 infants with Down syndrome at birth and identified 10 cardinal signs that were commonly present. Excess skin at the base of the neck was noted in 80% of children at birth. He noted, however, especially with regard to the facial appearance, that skin edema may be a factor and that prematurely born infants have a less pasty and almost normal skin. This suggests that skin thickening may be less apparent in early fetal life. Stephens and Shepard[8] studied 13 abor-

tuses with amniocentesis-verified trisomy 21 ranging in gestational age from about 17 to 25 weeks and compared them with normal aborted fetuses of similar size. They used Hall and other authors as a source of possible signs that might be detectable in such a young fetus. They found that only the simian crease, clinodactyly, septal heart defects, and small size were relatively common features in the trisomy 21 fetuses as compared with the normal control fetuses. They stated that the other features noted in term infants were less useful for identification of Down syndrome in early pregnancy.

With our autopsy review we were able to confirm that nuchal thickening can be present in the second trimester, but our 18% incidence is not as high as the 80% and 100% incidences noted in Down syndrome infants at term by Hall[1] and Fried[2] or the 36% (four of 11) found in the ultrasonographic series.[4]

At ultrasonography of chromosomally normal fetuses in the second trimester we found nuchal thickening of over 5 mm more commonly than the incidence of one per 1694 that has been reported. In our retrospective survey we found six of 28 (21%) with a false positive finding. Prospectively, we were able to make the nuchal measurement exceed 5 mm in every instance (seven of seven) when we angled the transducer caudally from Reid's baseline. Examination of a midsagittal dorsal ultrasonogram of a fetus explains this pseudothickening (Fig. 2). As the scan plane is maintained on the thalamus but is tilted to include the cerebellum and cisterna magna, the section will include an increasingly thick section of nuchal muscles and skin. This is of concern since the inexperienced observer may easily interpret this to be pathologic thickening, especially if he/she is conscientious enough to deliberately survey the nuchal area for this feature.

It is known that test properties such as sensitivity and specificity remain stable, irrespective of disease incidence. Unfortunately, this is not true for the more clinically useful positive and negative predictive values, which can undergo dramatic change if the same test is applied to populations that have a different incidence of disease. We can, however, calculate the expected performance in these circumstances.[6] As the incidence decreases, the positive predictive value also decreases. In the original ultrasonographic reports the incidence of Down syndrome was 1:155 and the predictive value of a positive test was 80%. However, in the general population where the incidence of Down syndrome is 1:660,[7] a positive test will predict disease in only 48% of instances. For pregnancy in women under age 30, the incidence is 1:1500 (Table I). Here a positive test will predict Down syndrome in only 20% of fetuses. This means that the other 80% or four of five fetuses with the finding will not have Down syndrome. As more information becomes available, these calculations may undergo significant change. Nevertheless, the 80% false positive rate in mothers under age 30 would suggest a more cautious approach than suggested by the previously published reports that urge that mothers be told that their fetus has a probability of having Down syndrome and have their options explained to them on the basis of only the ultrasonographic findings.

In summary, we feel that this potentially useful ultrasonographic sign of Down syndrome should be used with great caution. It is important to remember that the sign has been described only in the early second trimester and should not be used after 20 menstrual weeks. We were able to confirm that nuchal thickening does indeed occur in the second trimester in a small proportion of fetuses affected with Down syndrome. The ultrasonographic sign is as yet somewhat poorly described and it is possible to create false positive images in a significant proportion of chromosomally normal fetuses. In the normal low-risk obstetric population, the observation of the finding is more likely to identify a normal fetus than an abnormal one.

REFERENCES

1. Hall B. Mongolism in newborn infants. Clin Pediatr 1966;5:4-12.
2. Fried K. A score based on eight signs in the diagnosis of Down's syndrome in the newborn. J Ment Defic Res 1980;24:181-5.
3. Benacerraf BR, Barss VA, Laboda LA. A sonographic sign for the detection in the second trimester of the fetus with Down's syndrome. AM J OBSTET GYNECOL 1985;151:1078-9.
4. Benacerraf BR, Frigoletto FD, Laboda LA. Sonographic diagnosis of Down syndrome in the second trimester. AM J OBSTET GYNECOL 1985;153:49-52.
5. Department of Clinical Epidemiology and Biostatistics, McMaster University Health Sciences Centre. How to read clinical journals. II. To learn about a diagnostic test. Can Med Assoc J 1981;124:703-10.
6. Stempel LE. Eenie, meenie, minie, mo . . . what do the data really show? AM J OBSTET GYNECOL 1982;144:745-52.
7. Smith WD. Major problems in clinical pediatrics. Recognizable patterns of human malformation, vol 7. 3d ed. Philadelphia: WB Saunders, 1982:11-3.
8. Stephens TD, Shepard TH. The Down Syndrome in the fetus. Teratology 1980;22:37-41.

Clinical high-risk designation does not predict excess fetal-maternal hemorrhage

Paul M. Ness, M.D., Michael L. Baldwin, M.T.(A.S.C.P.), S.B.B., and Jennifer R. Niebyl, M.D.
Baltimore, Maryland

During a 5-year period, an enzyme-linked antiglobulin test was used to screen and quantitate fetal-maternal hemorrhage in 789 consecutive D-negative mothers who were delivered of D-positive babies. Six hundred seventy-two patients (85.2%) had no detectable fetal-maternal hemorrhage, and 117 patients (14.8%) had a detectable fetal-maternal hemorrhage. Eight of the 789 (1%) had a fetal-maternal hemorrhage >30 ml and required more than one vial of Rh immune globulin. Two patients with fetal-maternal hemorrhage of 29 and 30 ml also received additional Rh immune globulin. Each case was reviewed for the presence of high-risk features that are thought to predict patients at risk for fetal-maternal hemorrhage. Patients having a cesarean section or complicated vaginal delivery were considered to be in a group at high risk for fetal-maternal hemorrhage, while those with a spontaneous vaginal delivery were considered not to be at risk for fetal-maternal hemorrhage. Thirty-two of 237 patients (13.5%) in the risk group and 82 of 552 patients (15.3%) in the group not at risk had detectable fetal-maternal hemorrhage. The incidence of fetal-maternal hemorrhage for these two groups was not statistically different ($p > 0.50$ by χ^2 analysis). If only patients in the risk group had been screened for fetal-maternal hemorrhage, then five of 10 (50%) who required more than one vial of Rh immune globulin would have been undertreated and at risk for developing anti-D antibodies. In addition, newborn birth weight, Apgar scores, and hematocrits were examined for 13 cases of fetal-maternal hemorrhage of ≥21 ml, and none of these characteristics could be used to predict patients at risk for fetal-maternal hemorrhage. Therefore, no maternal or newborn characteristics could be identified that would reliably predict patients at risk for fetal-maternal hemorrhage. We conclude that all D-negative patients with D-positive babies should continue to be screened for fetal-maternal hemorrhage to identify those patients requiring more than one vial of Rh immune globulin. (Am J Obstet Gynecol 1987;156:154-8.)

Key words: High-risk pregnancy, fetomaternal hemorrhage, Rh immune globulin, enzyme-linked antiglobulin test

The American College of Obstetrics and Gynecology has recommended that screening for excess postpartum fetal-maternal hemorrhage in D-negative mothers who are delivered of D-positive infants be performed only for high-risk pregnancies.[1] This policy has raised much debate concerning the efficacy of screening only selected high-risk pregnancies.[2-4] Previously, a report from our institution examining postpartum blood samples from 186 D-negative mothers with D-positive babies during a 1-year period demonstrated that no clinical or historical factors could predict the patients at risk for any detectable fetal-maternal hemorrhage.[5] This report extends these findings to 789 patients studied prospectively during a 5-year period and examines the risk factors associated with fetal-maternal hemorrhage requiring more than one vial of Rh-immune globulin.

From the Immunohematology Division, Department of Laboratory Medicine, and the Department of Obstetrics and Gynecology, The Johns Hopkins Hospital.
Received for publication June 5, 1986; accepted August 11, 1986.
Reprint requests: Paul M. Ness, M.D., Department of Laboratory Medicine, The Johns Hopkins Hospital, 600 North Wolfe St., Baltimore, MD 21205.

Material and methods

The patients tested were D-negative mothers without anti-D antibodies who were delivered of Rh-positive babies at The Johns Hopkins Hospital from July 1980 through July 1985. No patients were excluded from this prospective study. Clotted blood specimens from the mother and baby were tested for ABO and D, including Du, by standard test tube methods.[6] A postpartum maternal blood sample collected in ethylenediaminetetraacetic acid (EDTA) was used to evaluate fetal-maternal hemorrhage by the enzyme-linked antiglobulin test, which has been previously described in detail.[7, 8] The test involves incubation of anti-D antibodies with the postpartum maternal EDTA blood sample, then the addition of an anti–immunoglobulin G antibody, which is conjugated with the enzyme alkaline phosphatase, and finally the addition of the substrate *p*-nitrophenyl phosphate, which is converted to a yellow

Table I. Fetal-maternal hemorrhage detected by the enzyme-linked antiglobulin test

Fetomaternal hemorrhage (fetal whole blood)	*Patients in current 5 yr study**		*Patients in previous 1 yr study[5]*	
	No.	*%*	*No.*	*%*
0 ml	672	85.2	166	89.2
0-10 ml	65	8.2	13	7.0
11-20 ml	39	5.0	5	2.7
21-30 ml	5	0.6	2	1.1
>30 ml	8	1.0	0	0.0
Total	789	100.0	186	100.0

*Includes the patients in the previous 1-year study.

Table II. Comparison of fetal-maternal hemorrhage with type of delivery

	Detectable fetomaternal hemorrhage						*No detectable fetomaternal hemorrhage*	
					Total			
	0-10 ml	*11-20 ml*	*21-30 ml*	*>30 ml*	*No.*	*%*	*No.*	*%*
Spontaneous delivery	48	30	3	4	85	73	467	70
Cesarean section	12	5	2	3	22	19	157	23
Complicated vaginal delivery	5	4	0	1	10	8	48	7
Total	65	39	5	8	117	100	672	100
ABO compatible					92	79	524	78
ABO incompatible					25	21	144	22
Total					117	100	668*	100

*Four cord blood types unavailable.

pigment for reading in a spectrophotometer. The blood sample of the patient is compared with a standard curve that contains 0%, 0.25%, 0.5%, and 1% of Rh-positive red blood cells, which corresponds to a fetal-maternal hemorrhage in a 70 kg mother (5000 ml blood volume) of 0, 12.5, 25, and 50 ml of whole blood.

Obstetric charts and a delivery suite log were reviewed to determine the mode of delivery. The mode of delivery was classified into one of three categories: spontaneous vaginal delivery, cesarean section, or complicated vaginal delivery. Delivery was considered complicated when forceps were used, multiple births occurred, abnormal third-trimester bleeding was noted, or fetal death was diagnosed in utero.

Results

Table I shows the fetal-maternal hemorrhage detected in 789 postpartum samples from Rh-negative mothers delivered of Rh-positive babies. Six hundred seventy-two of the 789 samples (85.2%) had no detectable fetal-maternal bleeding. One hundred seventeen of the 789 samples (14.8%) had a detectable fetal-maternal hemorrhage, and only eight of the 789 (1.0%) had bleeding >30 ml. Two samples in the 21 to 30 ml category had a fetal-maternal hemorrhage >25 ml, one of 29 ml and one of 30 ml. These two patients received an additional vial of Rh immune globulin. Table I also shows results from the previous 1-year study as compared with the 5-year study of this report.

Table II shows the number and total percentage of postpartum samples categorized by the amount of fetal-maternal hemorrhage and compared with the type of delivery. The percentage of patients in the three categories of delivery (spontaneous vaginal delivery, cesarean section, and complicated vaginal delivery) was similar for both the detectable and not detectable fetal-maternal hemorrhage groups, respectively: spontaneous vaginal delivery, 73% versus 70%; cesarean section, 19% versus 23%; and complicated vaginal delivery, 8% versus 7%. Table II also shows the number and percentage of babies that were ABO compatible or incompatible with the maternal ABO type. For those with a detectable fetal-maternal hemorrhage and those with no detectable fetal-maternal hemorrhage, the percentages are similar: ABO compatible, 79% versus 78%; ABO incompatible, 21% versus 22%.

Table III compares the amount of fetal-maternal hemorrhage for each type of complicated vaginal delivery or cesarean section. These data show that for any one high-risk group, the majority of patients have no detectable fetal-maternal hemorrhage. For all of these patients, only 32 of 237 (13.5%) had any detectable fetal-maternal hemorrhage. This is not statistically different from the incidence of fetal-maternal hemor-

Table III. Fetomaternal hemorrhage compared with type of complicated vaginal delivery or cesarean section

	Detectable fetomaternal hemorrhage (total n = 32)				*No detectable fetomaternal hemorrhage (total n = 205)*	*No. of patients with detectable fetomaternal hemorrhage per total no. of patients for each type of delivery*	
	0-10 ml	*11-20 ml*	*21-30 ml*	*>30 ml*		*No.*	*%*
Use of forceps	5	3	—	—	36	8/44	18
Multiple births	—	—	—	1	2	1/3	33
Fetal death	—	1	—	—	9	1/10	10
Ectopic pregnancy	—	—	—	—	1	0/1	0
Cesarean section	12	5	2	3	157	22/179	12
Total						32/237	13.5

Table IV. Clinical summary of 13 patients with fetomaternal hemorrhage of ≥21 ml

	Mother				*Newborn infant*				
Case No.	*Fetomaternal hemorrhage (ml of whole blood)*	*Vial of Rh immune globulin given*	*ACOG-defined risk group*	*Parity*	*Mode of delivery*	*Birth weight (gm)*	*Apgar score*		*Hematocrit (%)*
							1 min	*5 min*	
1	21	1	No	2-0-0-2	SVD	3330	9	9	58
2	27	1	Yes	0	PROM, C/S	2180	8	9	65
3	44	2	Yes	1-0-0-1	PROM, C/S	2560	9	9	56
4	37	2	Yes	5-0-0-5	AP, C/S	3960	6	9	75*
5	24	1	No	0-0-2-0	SVD	3340	9	9	61
6	168	8	Yes	0	PROM, C/S	2690	6	8	29†
7	36	2	No	2-0-1-2	SVD‡	2390	7	9	58
8	30	2	No	3-0-1-2	SVD‡	3250	9	9	48
9	65	3	Yes	2-0-0-2	SVD (twins)				
					Twin A	2260	8	9	55
					Twin B	2340	6	7	57
10	32	2	No	1-1-0-1	SVD‡	4190	8	8	50
11	36	2	No	0	SVD‡	3870	7	9	63
12	34	2	No	3-1-0-4	SVD‡	3780	7	8	47
13	29	2	Yes	3-0-0-3	PP, C/S	1480	6	7	33§

ACOG = American College of Obstetricians and Gynecologists; SVD = spontaneous vaginal delivery; PROM = premature rupture of membranes; C/S = cesarean section; AP = abruptio placentae; PP = placenta previa.

*Partial exchange transfusion for polycythemia.

†Transfusion for anemia attributed to large fetomaternal hemorrhage.

‡Spontaneous delivery of placenta.

§Premature infant born at about 30 weeks, with transfusion for anemia.

rhage for patients who had spontaneous vaginal delivery without any complications (Table II), where 85 of 552 (15.8%) had a detectable fetal-maternal hemorrhage ($p > 0.50$).

Table IV provides a summary of all 13 patients who had a fetal-maternal hemorrhage of ≥21 ml. Seven of these patients had a spontaneous vaginal delivery without any complicating factors, one patient had twins, and five patients had a cesarean section. The five patients with spontaneous vaginal delivery and bleeding >30 ml all had spontaneous delivery of the placenta. Manual removal of the placenta was not a cause of fetal-maternal hemorrhage in these patients. Birth weight, Apgar scores, and hematocrits were examined for each newborn infant to see if any of these would be indicators of fetal-maternal hemorrhage. Neonatal anemia might have suggested large amounts of fetal-maternal hemorrhage in only two cases, Case No. 13 (hematocrit 33%) at 30 weeks' gestational age and Case No. 6 (hematocrit 29%) with an unusually large fetal-maternal hemorrhage of approximately 168 ml. As shown in Table IV, five of the 10 patients receiving an additional vial of Rh immune globulin would have been missed if only risk groups defined by the American College of Obstetricians and Gynecologists were screened for excessive fetal-maternal hemorrhage.

Comment

The initial report from our hospital used enzyme-linked antiglobulin test to detect and measure fetal-

maternal hemorrhage in 186 patients during a 1-year period.[5] This report extends the study to 789 patients during a 5-year period and includes the 186 patients previously described. The percentage of patients with and without detectable fetal-maternal hemorrhage is similar for the two time periods as shown in Table I. The current study shows that 117 patients (14.8%) had a detectable fetal-maternal hemorrhage and that eight patients (1%) had a fetal-maternal hemorrhage >30 ml. This is similar to results in previous studies that used the acid-elution stain to quantitate fetal-maternal hemorrhage and showed an overall incidence of detectable bleeding to be 14.5% with 1% >30 ml.[9]

The American College of Obstetrics and Gynecology has stated that routine screening for excess fetal-maternal hemorrhage is not recommended but should, however, be performed for all high-risk pregnancies.[1] The American College of Obstetricians and Gynecologists defines high risk to be circumstances such as abruptio placentae, placenta previa, cesarean delivery, intrauterine manipulation (as with twins), or manual removal of the placenta. This report demonstrates that these risk factors are not accurate predictors of any detectable fetal-maternal hemorrhage or, more important, excess fetal-maternal hemorrhage requiring more than one vial of Rh immune globulin. In this study, 237 patients, or 30% of the total, were found to be in an American College of Obstetricians and Gynecologists risk category, either cesarean section or complicated vaginal delivery. However, only 32 of these 237 patients (13.5%) had a detectable fetal-maternal hemorrhage, which is not significantly different from the incidence in the group that was considered not to be at risk, where 82 of 552 patients (15.3%) had a detectable fetal-maternal hemorrhage ($p > 0.50$). These data show that being in an American College of Obstetricians and Gynecologists risk category is not a predictor of fetal-maternal hemorrhage.

Table IV provides a summary of all 13 patients found to have a fetal-maternal hemorrhage of ≥21 ml. If the criteria of the American College of Obstetricians and Gynecologists had been used to determine the population of patients requiring screening for fetal-maternal hemorrhage, four of the eight with a fetal-maternal hemorrhage >30 ml would have been missed and at risk for forming anti-D antibodies because of an inadequate dose of Rh immune globulin. In addition, two of the five patients with fetal-maternal hemorrhage between 21 and 30 ml had bleeding quantitated by the enzyme-linked antiglobulin test to be 29 and 30 ml. Since the reproducibility of the enzyme-linked antiglobulin test is ± 10%, each of these patients was given a second vial of Rh immune globulin. One of these patients was in a risk category (Case No. 13) and the other was not (Case No. 8). Again, if only the patients in risk categories were screened for excess fetal-maternal hemorrhage, then one of these two patients would have been at risk for alloimmunization and subsequent affected pregnancies. Therefore, in this study, a total of five of 10 patients (50%) would have been undertreated if only the criteria of the American College of Obstetricians and Gynecologists were followed.

We also investigated maternal parity, newborn birth weight, Apgar scores, and hematocrit for these 13 patients. These factors, taken singly or in combination, do not reliably predict excessive fetal-maternal hemorrhage. When the normal range for hematocrit[10] at birth is taken to be 53% to 65%, then only five of these newborn infants had hematocrits below the normal range (Cases 6, 8, 10, 12, and 13). Only in Cases 6 and 13 were the hematocrits markedly below normal. In Case 6, the hematocrit of 29% can be attributed to an unusually large fetal-maternal hemorrhage of approximately 168 ml. In Case 13, the fetal-maternal hemorrhage of 29 ml represents a large volume for a low birth weight infant (1480 gm) and accounts for the low hematocrit of 33%. However, as shown by Cases 3, 7, 9, and 11, the normal hematocrits give no indication of the excessive fetal-maternal hemorrhage requiring more than one vial of Rh immune globulin.

In summary, this study shows that there are no reliable indicators to predict patients at risk for fetal-maternal hemorrhage, or specifically at risk for excess fetal-maternal hemorrhage. Following the policy suggested by the American College of Obstetricians and Gynecologists would have resulted in half of the patients (five of 10) in this study being undertreated and at risk for developing anti-D antibodies. Simple, sensitive, and inexpensive methods exist to screen routinely for excessive fetal-maternal hemorrhage. The enzyme-linked antiglobulin test used in this study has the advantage of screening and quantitating with one procedure. The rosette technique as described by Sebring and Polesky[11] is a simple and inexpensive screening procedure that, if positive, can then be followed by a quantitative procedure. In the near future, other techniques that are sensitive, objective, and automated can be adapted to meet the medical requirements for fetal-maternal hemorrhage screening.

We therefore conclude that there are no medical or economic reasons not to screen all D-negative mothers delivered of D-positive babies for excess fetal-maternal hemorrhage. The availability of acceptable screening procedures would suggest potential liability if a patient develops anti-D antibodies in the absence of adequate screening. Finally, we would question whether any criteria for screening could be adopted that would result in screening of all indicated patients. Unless the criteria for which patients to screen are rigidly enforced and a suitable monitoring system can be implemented, many

patients will not receive screening even if they meet the criteria.

REFERENCES

1. Prevention of Rho(D) isoimmunization. Washington, DC: American College of Obstetricians and Gynecologists, 1984:79; Technical Bulletin.
2. Kolins J. The changing opinions of ACOG regarding the prevention of Rho(D) isoimmunization. Pathologist 1985; 39:37.
3. Schmidt PJ. Changing opinion of ACOG: is there a conflict? Pathologist 1985;39:5.
4. Debate waged over ACOG change in Rho(D) isoimmunization policy. Blood Bank Week 1985 July:3.
5. Ness PM. The assessment of fetal-maternal hemorrhage by an enzyme-linked antiglobulin test for Rh immune globulin recipients. AM J OBSTET GYNECOL 1982;143: 788-92.
6. Widman F, ed. Technical manual. 9th ed. Arlington, Virginia: American Association of Blood Banks.
7. Riley JZ, Ness PM, Taddie SJ, Barrasso C, Baldwin M. The detection and quantitation of fetal-maternal hemorrhage utilizing an enzyme linked antiglobulin test (ELAT). Transfusion 1982;22:472-4.
8. Leikola J, Perkins HA. Enzyme linked antiglobulin test: an accurate and simple method to quantify red cell antibodies. Transfusion 1980;20:138.
9. Lloyd LK, Miya F, Herbertson RM, Kochenour NK, Scott JR. Intrapartum fetomaternal bleeding in Rh negative women. Obstet Gynecol 1980;56:285.
10. Klaus MH, Fanaroff AA. Care of the high risk neonate. Philadelphia: WB Saunders, 1973.
11. Sebring ES, Polesky HF. Detection of fetal-maternal hemorrhage in Rh immune globulin candidates. Transfusion 1982;22:468-71.

Endometrial blood flow measured by xenon 133 clearance in women with normal menstrual cycles and dysfunctional uterine bleeding

Ian S. Fraser, M.D., Gay McCarron, R.N., Brian Hutton, M.Sc., and Daniel Macey, Ph.D.
Sydney and Camperdown, Australia, and Oxford, England

Endometrial blood flow was measured through the menstrual cycle in nonpregnant women (28 studies of 17 women with normal menstrual cycles and 32 studies of 20 women with dysfunctional uterine bleeding) with use of a clearance technique in which 100 to 400 μCi of the γ-emitting isotope, xenon 133 in saline solution was instilled into the uterine cavity. The mean (± SEM) endometrial blood flow in normal cycles was 27.7 ± 2.6 ml/100 gm/min, with a significant elevation in the middle to late follicular phase, followed by a substantial fall and a secondary slow luteal phase rise that was maintained until the onset of menstruation. There was a significant correlation between plasma estradiol levels and endometrial blood flow in the follicular but not the luteal phase. Blood flow patterns in women with ovulatory dysfunctional bleeding were similar to normal, except for a significantly lower middle follicular rate. Women with anovulatory dysfunctional bleeding exhibited exceedingly variable flow rates. (AM J OBSTET GYNECOL 1987;156:158-66.)

Key words: Endometrium, blood flow, menstrual cycle, dysfunctional uterine bleeding, xenon clearance

Several investigators have devised different approaches to the measurement of human myometrial and endometrial blood flow with use of inert gas clearance.[1-5] Most studies have concentrated on myometrial blood flow, and virtually nothing is known about absolute changes in human endometrial blood flow during normal or abnormal menstrual cycles.

The only previous study that has used a technique capable of giving a measure of the absolute rate of blood flow in human endometrium demonstrated a fall in flow when in the standing position compared with the supine one.[3] These investigators used a technique of intrauterine xenon 133 clearance, which has been explored and validated further by us.[6]

Preliminary information on relative changes in endometrial blood flow during the menstrual cycle has

From the Department of Obstetrics and Gynaecology, University of Sydney, the Department of Nuclear Medicine, Royal Prince Alfred Hospital, and the Department of Radiation Physics, The Churchill Hospital.
Received for publication July 15, 1986; revised August 8, 1986; accepted August 14, 1986.
Reprint requests: Ian S. Fraser, M.D., Department of Obstetrics and Gynaecology, University of Sydney, Sydney, New South Wales, 2006 Australia.

Table I. Clinical data on 17 women with normal menstrual cycles who underwent endometrial blood flow studies

Subject No.	*Age (yr)*	*Cycle length (day)*	*Days since last menstrual period*	*Plasma steroid levels*		*Endometrial history*	*Idealized cycle day*
				Estradiol (pg/ml)	*Progesterone (ng/ml)*		
C1	41	25-28	7	555	<1	P	10
C2	39	30-35	7	115	<1	P	5
C3	28	26-33	12	620	<1	LP	12
C4	37	28-31	4	–	–	P	4
C5	25	28-35	23	160	14.7	MS	22
C6	36	30-35	20	185	9.8	ES	18
C7	32	24-28	8	285	<1	P	10
C8	37	28-35	10	255	<1	P	9
C9	39	29-32	26	410	15.2	–	24
			24	365	12.6	MS	21
C10	30	27-32	25	290	8.5	–	23
			5	115	<1	–	5
			11	345	<1	–	11
C11	32	28-34	14	490	<1	–	12
C12	28	27-30	26	290	6.1	–	26
			18	360	10.9	–	19
C13	31	24-29	5	385	<1	–	8
			1	135	1.1	–	1
			3	150	<1	–	3
C14	35	27-29	14	335	<1	–	14
			9	220	<1	–	8
			28	105	1.3	–	27
C15	30	30-34	15	200	1.8	–	15
			29	265	5.3	–	27
			7	155	<1	–	6
C16	21	27-32	17	330	2.1	–	15
C17	28	26-30	25	265	12.0	–	25
			16	290	4.6	–	16

P = Proliferative; LP = late proliferative; ES = early secretory; MS = middle secretory.

been presented by Markee[7] from his work on endometrial ocular autotransplants in rhesus monkeys and by Prill and Götz[8] using a heat clearance technique in women. Neither of these methods can give a measure of absolute rates of flow, and the heat clearance technique cannot be accurately calibrated from day to day.

This report describes the first study of changes in absolute blood flow in human endometrium at different stages of the normal menstrual cycle and in a group of women with dysfunctional uterine bleeding.

Material and methods

Endometrial blood flow measurement was carried out with use of an intrauterine ^{133}Xe clearance technique. The theoretic considerations of inert gas clearance of this type from tissue have been well described and evaluated by Kety[9] and have been applied by a number of investigators to the study of human myometrial[1-5] and endometrial blood flow.[3] Testing of theoretic considerations has been described more fully (Fraser IS, McCarron G, Hutton BF, Macey D. An evaluation of two inert gas clearance techniques for measurement of endometrial blood flow in women. Unpublished observations).

^{133}Xe (100 to 400 μCi) was instilled through a fine and moderately pliable catheter (Portex Instruments, Hythe, Kent, England; size 4FG, external diameter of 1.34 mm) in a small volume (0.05 to 0.1 ml) of normal saline solution into the uterine fundus. Steady uptake into endometrial tissue and clearance into the venous circulation are directly related to endometrial blood flow. Over 90% of the gas is then eliminated from the body in one passage through the lungs. The removal of radioactivity from the uterine cavity was detected by an external gamma camera (Searle Pho-Gamma 4, with a 28 cm field and a high sensitivity collimator) and recorded and analyzed with use of a computer interfaced with the gamma camera. The clearance curves were analyzed into two or three single exponential components on log-linear scales. The flow rates in each compartment were calculated from the following equation, assuming a tissue/blood partition coefficient for ^{133}Xe of 1.0[10] and a specific gravity for endometrium of 1.0:

$$F = 100k \text{ ml}/100 \text{ gm/min}$$

where $k = 0.693 \div t_{1/2}$ and $t_{1/2}$ = the half-time of the exponential in minutes. The relative sizes of each of

Table II. Clinical data on 20 women with dysfunctional uterine bleeding who underwent endometrial blood flow studies

Subject No.	Age (yr)	Cycle length (day)	Days since last menstrual period	Plasma steroid levels		Endometrial histology	Type of dysfunctional uterine bleeding	Idealized cycle day
				Estradiol (pg/ml)	Progesterone (ng/ml)			
AD1	46	14-50	22	600	<1	LP	ANOV	(22)
AD2	43	20-60	16	380	<1	LP	ANOV	(16)
AD3	45	28-50	20	555	<1	CGH	ANOV	(20)
AD4	41	26-60	2	–	–	–	ANOV	(2)
			9	540	<1	–		(9)
AD5	29	25-35	23	490	<1	–	ANOV	(23)
			9	720	<1	–		(9)
AD6	31	18-40	26	205	<1	–	ANOV	(26)
OD1	39	28-35	31	335	4.6	LS	OV	27
OD2	42	21-30	13	–	–	ES	OV	17
OD3	36	24-30	1	–	–	M	OV	1
OD4	35	27-40	28	340	24.7	MS	OV	21
OD5	31	28-35	12	195	<1	P	OV	8
OD6	36	29-35	9	135	<1	–	OV	5
			12	200	<1	–		9
			28	225	10.8	–		23
OD7	34	25-28	8	670	<1	–	OV	10
			21	180	7.6	–		25
			24	145	4.9	–		26
OD8	31	26-35	12	420	<1	–	OV	12
			14	135	6.3	–		16
			35	105	2.1	–		27
OD9	40	27-30	11	455	<1	–	OV	11
			9	390	<1	–		9
OD10	38	29-35	6	90	<1	–	OV	4
OD11	29	28-31	26	145	8.3	–	OV	25
OD12	34	27-32	15	430	<1	–	OV	11
			14	275	10.4	–		15
			24	280	14.5	–		24
OD13	41	26-31	10	645	<1	–	OV	11
			19	295	11.8	–		19
OD14	37	21-25	5	585	<1	–	OV	10

ANOV = Anovulatory; OV = ovulatory; P = proliferative; LP = late proliferative; ES = early secretory; MS = middle secretory; LS = late secretory; M = menstrual; CGH = cystic glandular hyperplasia.

the compartments as a percentage of total tissue mass (m′% and m″%) were calculated by back extrapolation of the exponents to time zero to obtain the initial distribution of counts to each compartment (Ao′ and Ao″):

$$\frac{Ao'}{Ao''} = \frac{F' \times m'\%}{F'' \times m''\%}$$

and the mean endometrial flow (F) from the relation:

$$F = (F' \times m'\%) + (F'' \times m''\%)$$

Experimental data from ewes[6] indicated that the mean flow with use of both compartments gave a more accurate assessment of total endometrial flow than use of any single compartment flow on its own. However, there is still considerable controversy as to the significance of different compartments, which are therefore reported separately here.

Analysis was carried out with use of a computer (Digital Equipment Corporation, Model PDP 11/34 Gamma 11) and modified iterative function minimization program[11] for fitting a multiexponential curve by the least squares method. The formula for the washout curve was as follows:

$$A(t) = Ao' \exp(-k't) + Ao'' \exp(-k''t)$$

where A is the activity at the time t, and Ao′, Ao″, k′, and k″ are the unknown constants to be estimated for a two- or three-component exponential.

The analysis of the curves obtained assumes a bimodal distribution of blood flow into two or three homogeneously perfused compartments that are arranged in parallel. After conversion to a log-linear scale, analysis involves initial identification of the slowest component (after background subtraction), which is then subtracted from the original raw data curve to leave the faster component. In occasional experiments an ultrafast third component was identified when the second component was subtracted. The existence of different components or compartments points to the existence of portions of endometrial tissue with different blood flow rates. The influence of small air bubbles

Table III. Endometrial blood flow in 17 women with normal menstrual cycles

		Component 1			Component 2			Component 3			
Subject No.	*Idealized cycle day*	*t½ (min)*	*Flow (ml/100 gm/min)*	*Size (%)*	*t½ (min)*	*Flow (ml/100 gm/min)*	*Size (%)*	*t½ (min)*	*Flow (ml/100 gm/min)*	*Size (%)*	*Mean flow (ml/100 gm/min)**
C1	10	36.6	1.9	4	1.2	55.7	96	–			53.5
C2	5	>100	<1	<1	4.5	15.5	100	–			15.5
C3	12	17.9	3.9	2.5	1.4	49.1	97.5	–			48.0
C4	4	84.4	<1	1.2	6.1	11.4	98.8	–			11.3
C5	22	28.3	2.4	5	1.8	37.5	95	–			35.8
C6	18	15.7	4.4	14	3.6	19.2	86	–			17.1
C7	10	21.2	3.3	3	3.3	21.0	40	1.4	49.5	57	36.8
C8	9	40.4	1.7	6	2.2	31.4	94	–			29.6
C9	24	>100	<1	<1	1.7	40.2	100	–			40.2
	21	29.9	2.3	12	1.8	38.6	88	–			34.3
C10	23	>100	<1	<1	2.3	29.9	100	–			29.9
	5	>100	>1	<1	2.6	27.0	100	–			27.0
	11	16.4	4.2	5	2.7	25.7	95	–			24.6
C11	12	>100	<1	<1	6.7	10.3	39	0.66	105.0	61	68.1
C12	26	26.1	2.6	<1	1.9	34.9	99+	–			34.7
	19	12.6	5.5	2.6	3.5	19.6	97.4	–			19.2
C13	8	11.6	6.0	17	2.0	34.0	83	–			29.2
	1	14.9	4.7	1.9	2.5	27.9	98.1	–			27.5
	3	27.6	2.5	14	4.9	14.1	86	–			12.5
C14	14	34.9	2.0	13	4.8	14.5	87	–			12.9
	8	87.2	0.8	1.5	2.5	27.1	98.5	–			26.7
	27	28.9	2.4	4	2.2	31.7	96	–			30.5
C15	15	31.7	2.2	5	4.1	17.0	95	–			16.3
	27	>100	<1	<1	2.8	25.1	100	–			25.1
	6	>100	<1	<1	3.1	22.1	100	–			22.1
C16	15	39.4	1.8	48	4.4	15.8	52	–			9.1
C17	25	18.2	3.8	6	2.6	26.7	94	–			25.3
	16	22.4	3.1	6	5.7	12.1	94	–			11.6

*Mean ± SEM = 27.7 ± 2.6.

in the injection, volume of injection fluid, and repeated installations on clearance curves have all been studied in detail and have not been found to interfere significantly with the flow measurements (Fraser IS, McCarron G, Hutton BF, Macey D. An evaluation of two inert gas clearance techniques for measurement of endometrial blood flow in women. Unpublished observations).

Subjects studied included 17 women with normal menstrual cycles (Table I) and 20 women with dysfunctional uterine bleeding (6 anovulatory) (Table II). These women were studied at different stages of the menstrual cycle, and 15 were studied on more than one occasion (seven normal cycles and eight with dysfunctional uterine bleeding). Dysfunctional uterine bleeding has been defined as excessively heavy, excessively prolonged, or excessively frequent uterine bleeding in the absence of recognizable pelvic or generalized medical disease or pregnancy.[12] All subjects enrolled into this study gave a convincing history of menorrhagia.

Almost all subjects also had plasma estradiol and progesterone levels measured at the time of blood flow studies, and most had several samples taken at other stages in the same cycle. All women kept an accurate note of data of each preceding and succeeding menstrual period, and some maintained basal body temperature and vaginal mucus charts. A combination of these pieces of information permitted dating of each blood flow measurement to an idealized 28-day menstrual cycle, except in those women with a history of anovulatory dysfunctional uterine bleeding.

Results

^{133}Xe washout half-times and supine endometrial blood flow calculations for each subject are summarized in Tables III and IV. The overall mean (±SEM) endometrial blood flow in women with normal cycles was 27.7 ± 2.6 ml/100 gm/min when all measurements were considered together. Control subjects and women with ovulatory and anovulatory dysfunctional uterine bleeding have been considered separately in Table V according to this stage of the cycle. Although numbers in each group are small, there does not appear to be much difference between women with normal menstrual cycles and those with ovulatory dysfunctional uterine bleeding (Figs. 1 and 2). However, blood flow in normal women in the middle follicular phase (days 8 to 12) was significantly higher than in the women with ovulatory dysfunctional uterine bleeding (39.6 and 25.2 ml/100 gm/min; $t = 2.274$, $p < 0.05$). One woman with

Table IV. Endometrial blood flow in 20 women with dysfunctional uterine bleeding

Subject No.	Idealized cycle day	Component 1			Component 2			Component 3			Mean flow (ml/100 gm/min)*
		t½ (min)	Flow (ml/100 gm/min)	Size (%)	t½ (min)	Flow (ml/100 gm/min)	Size (%)	t½ (min)	Flow (ml/100 gm/min)	Size (%)	
AD1	22	16.3	4.3	35	1.1	63.0	65	–			42.5
AD2	16	25.6	2.7	8	3.8	18.2	51	1.7	40.8	41	26.2
AD3	20	19.1	3.7	22	1.5	46.2	88	–	–		41.5
AD4	2	13.7	5.1	29	4.8	14.3	71	–			11.6
	9	14.4	4.8	14	2.5	28.2	86	–			24.9
AD5	23	20.4	3.4	2.8	1.7	42.0	97.2	–			40.9
	9	19.5	3.6	1.7	1.2	57.8	98.3	–			56.9
AD6	26	31.5	2.2	9	1.6	42.2	91	–			38.6
OD1	27	26.7	2.6	2	1.3	52.8	98	–			51.8
OD2	17	21.0	3.3	8	3.0	22.9	92	–			21.3
OD3	1	13.6	5.1	10	2.5	27.2	90	–			25.0
OD4	21	23.7	2.9	4.8	3.2	22.0	95.2	–			21.1
OD5	8	18.8	3.7	12	4.8	14.5	58	2.5	27.3	30	17.0
OD6	5	82.0	0.85	2.6	3.4	20.1	97.4	–			19.6
	9	20.0	3.5	11	2.9	23.6	50	2.1	33.6		25.3
	23	49.4	1.4	<1	1.7	40.8	99+	–			40.6
OD7	10	14.0	5.0	6	2.2	31.5	94	–			29.9
	25	>100	<1	<1	1.9	35.7	100	–			35.7
	26	29.4	2.4	<1	2.0	35.5	99	–			35.3
OD8	12	15.5	4.5	5	4.6	15.1	95	–			14.6
	16	15.2	4.6	43	5.5	12.6	57	–			9.2
	27	>100	<1	>1	2.4	29.4	100	–			29.4
OD9	11	18.9	3.7	7	3.1	22.7	93	–			21.4
	9	18.6	3.7	<1	4.3	16.3	99+	–			16.2
OD10	4	61.5	1.1	7	7.3	9.5	93	–			8.9
OD11	25	22.5	3.1	3	2.1	33.5	97	–			32.3
OD12	11	14.7	4.7	6	2.8	24.8	94	–			23.5
	15	>100	<1	<1	3.7	19.0	100	–			19.0
	24	100	1	1	3.0	23.4	100	–			23.4
OD13	11	44.4	1.6	1.4	2.3	30.5	98.6	–			30.1
	19	12.0	5.8	10	6.3	11.0	42	2.3	29.7	48	19.5
OD14	10	>100	<1	<1	1.4	48.5	100	–			48.5

*Mean ± SEM = 28.2 ± 2.2.

Table V. Endometrial blood flow measurements at different stages of the menstrual cycle in women with normal cycles or dysfunctional uterine bleeding

	Early follicular days 1-7	Middle follicular days 8-12	Peri-ovulatory days 13-16	Early luteal days 17-21	Middle luteal days 22-25	Pre-menstrual days 26-28	Persistent follicular (anovulatory)
Normal cycles	19.3 (n = 6)	39.6 (n = 8)	12.5 (n = 4)	23.5 (n = 3)	32.8 (n = 4)	30.1 (n = 3)	–
Ovulatory dysfunctional uterine bleeding	17.8 (n = 3)	25.2 (n = 9)	14.1 (n = 2)	20.6 (n = 3)	33.0 (n = 4)	38.8 (n = 3)	–
Anovulatory dysfunctional uterine bleeding	11.6 (n = 1)	40.9 (n = 2)	–	–	–	–	37.9 (n = 5)

ovulatory dysfunctional uterine bleeding had a high rate of flow (51.8 ml/100 gm/min) on the day before menstruation, but others with dysfunctional uterine bleeding were in the same range as control subjects in the premenstrual period.

The number of women with anovulatory dysfunctional uterine bleeding was too small to make any comment except that exceedingly variable blood flows were recorded and these overlapped with normal levels.

Women with normal cycles and ovulatory dysfunctional uterine bleeding had an obvious cyclical variation in endometrial blood flow with high levels in the middle to late follicular phase which fell substantially around ovulation. A secondary rise occurred through the early and middle luteal phases and was maintained until the onset of menstruation. In two women (C13 and OD3) measurements were carried out just after the onset of premenstrual spotting and a few hours before the onset of full flow. In both these cases endometrial flow was still maintained at a fairly high level (27.5 and 25.0 ml/100 gm/min). Later in menstruation and during the early follicular phase flow was slower.

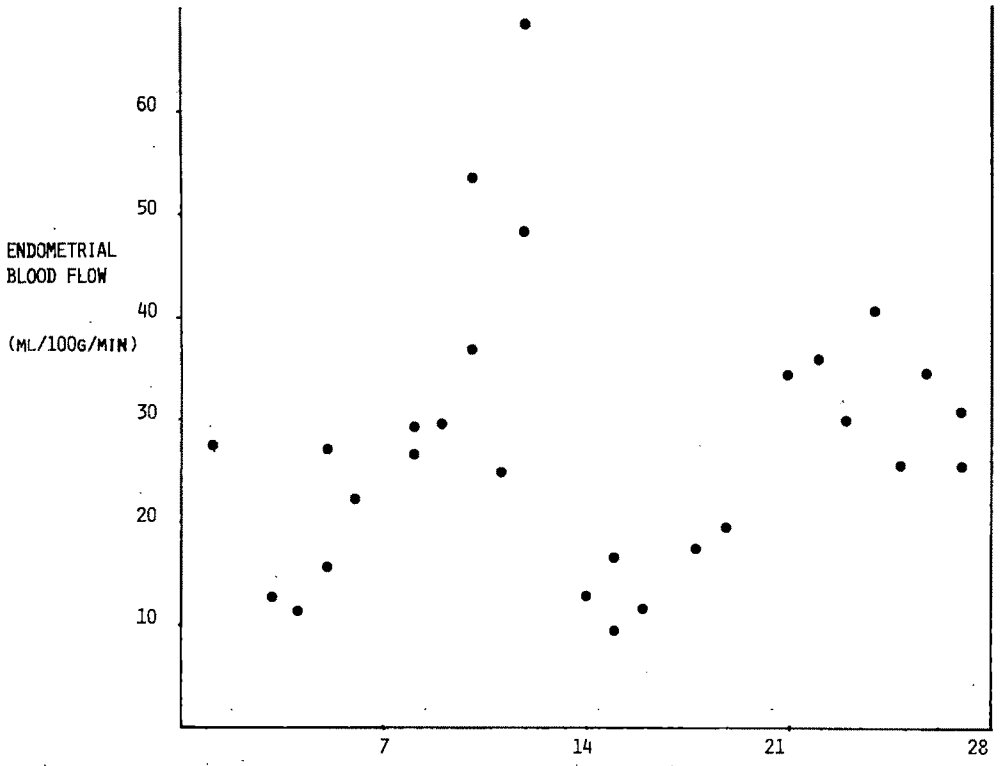


Fig. 1. Endometrial blood flow (ml/100 gm/min) measured using ^{133}Xe clearance at different stages of the menstrual cycle in women with normal cycles. Data have been adjusted to fit into an idealized 28-day menstrual cycle.

Only six examples of three component curves were recorded, which occurred in both dysfunctional uterine bleeding groups as well as in normal cycles. There did not appear to be any particular pattern to their occurrence, and mean flows were quite variable (17.0 to 68.1 ml/100 gm/min).

A clear correlation between plasma estradiol levels and endometrial blood flow was recorded for all studies undertaken when progesterone levels were <1 ng/ml ($r = 0.622$; $n = 31$) (Fig. 3) and for women with normal cycles when progesterone was <1 ng/ml ($r = 0.760$; $n = 13$). This correlation between plasma estradiol and endometrial blood flow was lost when progesterone was elevated (total subjects: $r = 0.016$; $n = 25$). No correlation between plasma progesterone levels and endometrial blood flow was found for luteal phase studies (total: $r = 0.073$; $n = 25$). This poor correlation was particularly obvious when endometrial blood flow did not fall substantially during the premenstrual period at a time when estradiol and progesterone were falling rapidly.

Comment

Most previous work on genital tract blood flow in nonpregnant women has been directed toward study of myometrium, myomas, endometrial cancer, amenorrhea, dysmenorrhea, and changes in posture, and no previous systematic studies have been carried out in normal and abnormal cycles or in relation to menstruation. This investigation has provided the first assessment of changes in absolute blood flow in human endometrium through the menstrual cycle, as well as in women with dysfunctional uterine bleeding.

Cyclical changes in endometrial blood flow have been clearly demonstrated, with high levels in the middle to late follicular phase, a large fall at around ovulation, and a secondary luteal phase rise that was maintained until menstruation. The preovulatory measurements showed a positive correlation between circulating levels of estradiol and endometrial blood flow. This correlation was lost during the luteal phase. The major fall in blood flow at around ovulation seemed to slightly precede the midcycle rise in progesterone. Unfortunately, the progesterone assays used in the study were not reported sufficiently accurately in the lowest part of the range to exclude a very small rise in progesterone in two subjects (C14 and OD8) who had low blood flow levels at days 12 and 14. Other factors than plasma estradiol and progesterone levels must influence endometrial blood flow, at least during some stages of the cycle.

In the latter part of the luteal phase when luteolysis was occurring, there was no evidence of any related fall in endometrial blood flow. In fact, blood flow was still

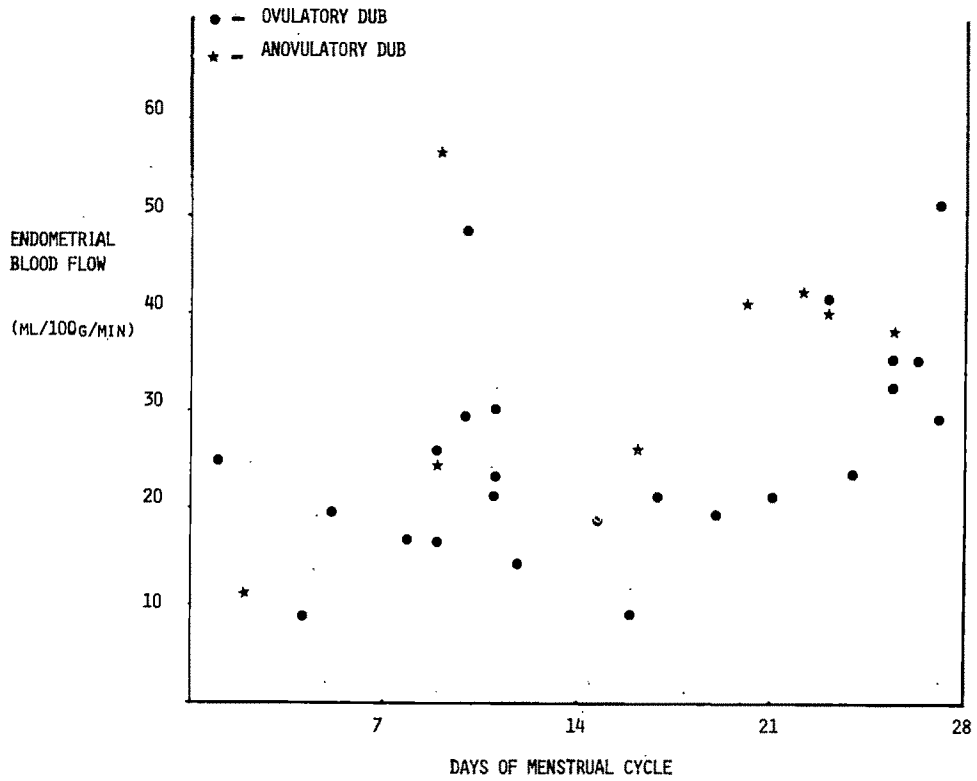


Fig. 2. Endometrial blood flow (ml/100 gm/min) measured using ^{133}Xe clearance in women with ovulatory and anovulatory dysfunctional uterine bleeding. Data for women with ovulatory dysfunctional uterine bleeding have been adjusted to fit into an idealized 28-day menstrual cycle.

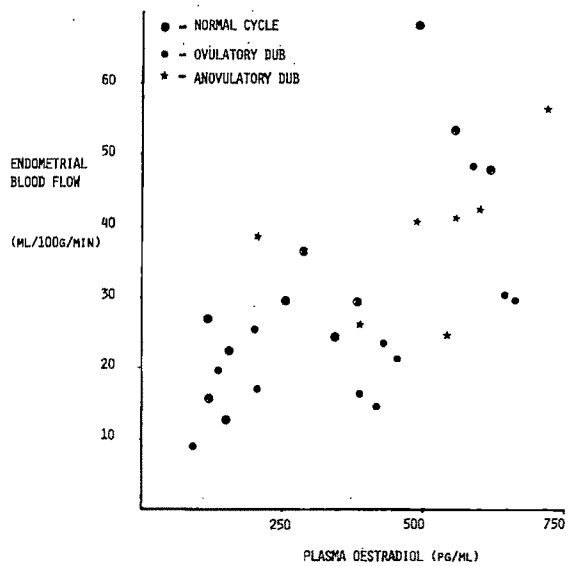


Fig. 3. Comparison of endometrial blood flow with plasma levels of estradiol in women with normal cycles and ovulatory or anovulatory dysfunctional uterine bleeding. Only follicular phase measurements (plasma progesterone level of <1 ng/ml) have been included.

relatively high (27.5 and 25.0 ml/100 gm/min) in two subjects who were experiencing premenstrual spotting and who began bleeding heavily within 3 to 4 hours.

Two previous studies have provided evidence of the occurrence of cyclical changes in endometrial blood flow during the menstrual cycle. Markee[7] studied endometrial autotransplants in the anterior chamber of the eye of rhesus monkeys and measured blood flow by a stroboscopic technique that assessed the speed of movement of red cells through stromal capillaries in millimeters per second. Prill and Götz[8] used a heat clearance probe technique to study cyclical changes in human endometrial blood flow in $10^{-4}cal \cdot cm^{-1} \cdot sec^{-1} \cdot °c^{-1}$, although this type of technique cannot be exactly recalibrated from one day to the next. It is also pertinent to note that an intrauterine probe may cause artificial changes in local blood flow. A comparison of the pattern of cyclical changes demonstrated by these two groups of investigators with the present ^{133}Xe study is shown in Fig. 4. All studies showed a preovulatory peak with a postovulatory fall and a secondary rise in the luteal phase, although clear differences are also apparent. The ^{133}Xe clearance technique demonstrated a much more marked periovulatory fall in endometrial flow with a very slow rise in the luteal phase. With the heat clearance technique a slow premenstrual rise in flow was also seen and this continued until days 2 to 3 of menstruation. In the rhesus monkeys a marked premenstrual fall in flow was seen. Much of our current understanding of the morphology of menstruating endometrium is based on the rhesus monkey studies of

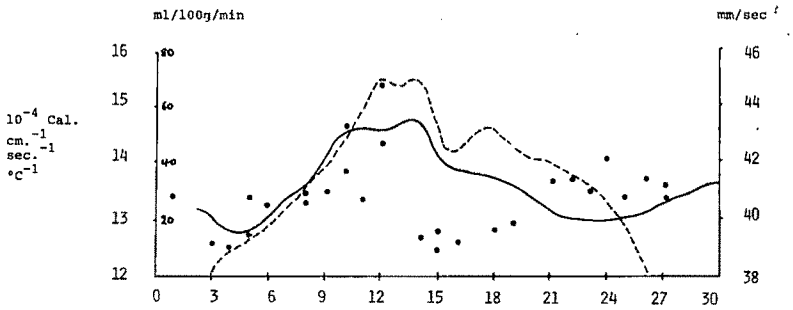


Fig. 4. Comparison of endometrial blood flow at different stages of the menstrual cycle measured by ^{133}Xe clearance in normal women (ml/100 gm/min) and compared with the data of Markee[7] in rhesus monkeys (mm/sec; *dotted line*) and Prill and Götz[8] using a heat clearance technique in women (10^{-4}cal · cm^{-1} · sec^{-1} · °C^{-1}; *continuous line*).

Markee,[13] especially with respect to the occurrence of dramatic vasoconstriction in the endometrium just before and at the onset of bleeding. Our study and that of Prill and Götz[8] suggest that this may not occur, at least to the same extent in humans, in which case the local coagulation mechanisms studied by Christiaens et al.[14] may play a more dominant role in the maintenance of hemostasis in the human uterus. Nevertheless, in pathologic conditions such as spasmodic dysmenorrhea other factors such as excessive uterine contractions may produce substantial falls in endometrial blood flow during menstruation.[15] There is also some histologic evidence that arteriolar vasoconstriction may sometimes occur in human late luteal phase endometrium.

Only one previous investigation has reported absolute flow measurements in human endometrium.[3] This study used a different technique of clearance analysis which may not always be appropriate for uterine blood flow studies.[5] However, this study does report the first measurements of absolute flow in human endometrium, and although the mean level (17.8 ml/100 gm/min; range, 13.5 to 29.0) is lower than the present study (27.7 ml/100 gm/min); range, 9.1 to 68.1), the range overlaps greatly. It is of interest that all the ^{133}Xe studies of blood flow in human (nonpregnant) myometrium are in the same range of 3 to 40 ml/100 gm/min. This is very similar to the range of measurements of endometrial flow, although it has been demonstrated in sheep that endometrial and myometrial flow may show large independent variations.[6] Myometrial blood flow in pregnancy is very much higher (77 ± 2.5 ml/100 gm/min; mean ± SEM).

Many aspects of endometrial function are poorly understood, and this applies particularly to endometrial blood flow. Blood flow is clearly important for nourishment of the endometrium and may vary greatly at different levels in the tissue and under the influence of different hormones. There is a need for a more systematic assessment of serial change in individual women, of changes during the cycle, and of changes in different hormonal states (for example, during exogenous hormonal treatment) as well as for a comparison with methods that describe different aspects of endometrial blood flow such as heat clearance[15] and krypton 85 clearance (Fraser IS, McCarron G, Hutton BF, Macey D. An evaluation of two inert gas clearance techniques for measurement of endometrial blood flow in women. Unpublished observations). This ^{133}Xe method is the only approach that could be used at present to assess the effect of hormones and other substances such as prostaglandins, prostaglandin inhibitors, catecholamines, histamines, angiotensin, and various other drugs on absolute changes in endometrial blood flow. Any or all of these may have implications for variations in blood loss at the onset of menstruation. Modern computer analysis techniques could be developed to provide more precise information about all these changes and a better knowledge of this poorly understood aspect of endometrial physiology and pathophysiology. It seems unlikely that techniques of this type will offer any early prospect of aiding or improving the precision of clinical diagnosis or management.

REFERENCES

1. Munck O, Lysgaard H, Pontonnier G, Lefevre H, Lassen NA. Measurement of blood flow through uterine muscle by local injection of 133-xenon. Lancet 1964;1:1421.
2. Jansson I. 133-Xenon clearance in the myometrium of pregnant and non-pregnant women. Acta Obstet Gynecol Scand 1969;48:302.
3. Secher NJ, Einer-Jensen N, Juhl B. Blood flow through the myometrium and endometrium in standing and supine women measured by intrauterine Xenon application. Am J Obstet Gynecol 1973;117:386.
4. Klingenberg I. Measurement of blood flow in human myometrium by local hydrogen clearance. Acta Obstet Gynecol Scand 1969;48:470.
5. Forssman L. Methods of calculating uterine blood flow from the washout curves of intraarterial and local injections of 133 Xenon. Acta Obstet Gynecol Scand 1975; 54:479.
6. Fraser IS, Brown BW, Mattner PE, Hutton BF. Measurements of endometrial blood flow in anaesthetised ewes by xenon-133 clearance and microsphere techniques. J Exp Physiol 1989;67:531

7. Markee JE. The relation of blood flow to endometrial growth and the inception of menstruation. In: Engle ET, ed. Menstruation and its disorders. Springfield, Illinois: Charles C Thomas, 1950:165.
8. Prill HJ, Götz F. Blood flow in the myometrium and endometrium of the uterus. AM J OBSTET GYNECOL 1961; 82:102.
9. Kety SS. The theory and applications of the exchange of inert gas at the lungs and tissues. Pharmacol Rev 1951;3:1.
10. Thorburn GD, Casey BH, Molyneux GS. Distribution of blood flow within the skin of the rabbit with particular reference to hair growth. Circ Res 1966;18:650.
11. Powell MJD. An efficient method for finding the minimum of a function of several variables without calculating derivatives. Comput J 1964;7:155.
12. Fraser IS. The dysfunctional uterus: dysmenorrhoea an dysfunctional uterine bleeding. In: Shearman RP, ec Clinical reproductive endocrinology. Edinburgh: Chu chill Livingstone, 1985:579.
13. Markee JE. Menstruation in intraocular endometri transplants in the rhesus monkey. Contrib Embryol Ca negie Inst 1940;28:219.
14. Christiaens GCML, Sixma JJ, Haspels AA. Hemostasis i menstual endometrium: a review. Obstet Gynecol Sur 1982;37:281.
15. Akerlund M, Bengtsson LP, Carter AM. A technique fo monitoring endometrial or decidual blood flow with a intrauterine thermistor probe. Acta Obstet Gynecol Scan 1975;54:469.

Routine hepatitis screening in adolescent pregnancies: Is it cost effective?

Ann M. Wetzel, M.D., and Donna S. Kirz, M.D.
Chicago, Illinois

In most obstetric centers routine serum screening for hepatitis B is not part of standard prenatal care. This study was designed to determine whether hepatitis B screening is cost effective for routine prenatal testing. In a prenatal population of 585 adolescents the cost-benefit ratio of hepatitis B screening was compared with that of routine syphilis screening. Eight positive results were detected, with a cost of $1755 per positive case. This compared favorably with the frequency and cost of detection of syphilis in the same population. Routine hepatitis B screening is recommended for consideration in similar prenatal populations. (AM J OBSTET GYNECOL 1987;156:166-9.)

Key words: Hepatitis, adolescents, pregnancy, cost-effective screening

During the past several decades, the development and widespread use of hepatitis B screening have increased an awareness of this serious disease. Programs have been initiated to screen all blood donors and thus prevent the spread of hepatitis B by blood transfusion. Another major route of transmission of the disease is vertical transmission from mother to infant in the perinatal period. In the United States this route has not yet received as much attention and investigation as has transmission by blood transfusion.

In some countries in Asia, hepatitis B is a major maternal health problem. As much as 10% of the population may be carriers.[1] Therefore screening for hepatitis B is part of routine prenatal care. In the United States, hepatitis B screening is routine for prenatal patients of Chinese and Southeast Asian extraction. Among other racial groups, guidelines are much les clear. Whether the disease is sufficiently common t justify routine prenatal screening has not yet been in vestigated.

This study was designed to answer that question. I an adolescent prenatal population we determined th incidence of hepatitis B and compared the cost of hep atitis B screening with that of the universally accepte routine VDRL screening.

Material and methods

Rush-Presbyterian-St. Luke's Medical Center is large private university hospital located on Chicago' West Side. There is a delivery service of approximatel 3200 women. To care for the health needs of pregnan adolescents, the Adolescent Family Center was estab lished. The Adolescent Family Center patients are pre dominantly inner-city, black, poor, and mostly on publi aid. Care is provided by a team of nurse-midwives social workers, and a nutritionist, with supervision o staff obstetricians.

From the Department of Obstetrics and Gynecology, Rush-Presbyterian-St. Luke's Medical Center.
Received for publication August 23, 1985; revised February 11, 1986; accepted August 14, 1986.
Reprint requests: Donna S. Kirz, M.D., Department of Obstetrics and Gynecology, Rush-Presbyterian-St. Luke's Medical Center, 1753 West Congress Parkway, Chicago, IL 60612.

Table I. Demographics of study groups

	Age (yr)		*Gravidity*		*Parity*		*Abortuses*		*Birth weight (gm)*		*1 min Apgar score*		*5 min Apgar score*	
Group	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Total	16.20	1.40	1.45	0.68	0.31	0.53	0.14	0.38	3074.37	538.36	7.70	1.87	8.62	1.24
Group 1	16.25	1.39	1.43	0.68	0.29	0.53	0.14	0.39	3043.96	510.22	7.74	1.85	8.66	1.21
Group 2	16.11	1.43	1.47	0.69	0.34	0.53	0.13	0.37	3124.19	579.28	7.65	1.92	8.54	1.28

Group 1: Patients presenting before 1983, screened with hepatitis B surface antigen alone. Group 2: Patients presenting after 1983, who underwent full serologic screening.

From 1981 to mid-1983, a hepatitis B surface antigen test was obtained for all patients initiating care at the Adolescent Family Center clinic. Because of the number of positive results obtained, it was decided to study hepatitis B in this population in greater depth. Accordingly, from late 1983 until February 1985 all patients were screened at the first visit with a hepatitis profile consisting of at least a hepatitis B surface antigen and a hepatitis B surface antibody. Additionally, since the beginning of 1984, hepatitis B e antigen and hepatitis B e antibody have also been evaluated.

Tests were performed either by Mason-Barron Laboratories or the laboratory of Rush-Presbyterian-St. Luke's Medical Center. Hepatitis B surface antigen was determined by an enzyme-linked immunosorbent assay or radioimmunoassay. Other antigen and antibody tests were performed by radioimmunoassay at Rush-Presbyterian-St. Luke's. All patients also had a rapid plasma reagin test performed at the initial prenatal visit. If this was positive, a fluorescent treponemal antibody absorption test was performed to confirm syphilis infection. A rapid plasma reagin test was also routinely done at 32 weeks and on admission to the hospital for delivery.

Any positive results obtained were reviewed by the attending obstetricians supervising the clinic and appropriate treatment was initiated. In the case of positive hepatitis B surface antigen results the nursery was informed so that treatment of the infant could be carried out.

The data obtained were analyzed by determining the incidence of positivity for both hepatitis and syphilis. The cost of tests was established by the laboratory at Rush-Presbyterian-St. Luke's Medical Center. Subgroups were analyzed by χ^2 analysis to examine the significance of presumed risk factors.

Results

Data were obtained for 585 patients. Of these, 361 presented before mid-1983 and composed the first group. The remaining 224 had the more complete hepatitis profile performed, including hepatitis B e antigen and hepatitis B e antibody (group 2).

The age range was 12 to 19 years; 85% were between 15 and 18. The mean and SD of age, gravidity, parity, and pregnancy outcome, as represented by fetal weight and Apgar scores, are given in Table I. It can be seen that the two groups differed very little. The population was overwhelmingly unmarried. Of those who gave a marital status 95.5% were unmarried. There were 94% black, 2% Caucasian, and 4% Hispanic women. These percentages were essentially the same in both groups.

Table II. Hepatitis profile and rapid plasma reagin test results in group 2

	Positive results	
Test	*Frequency*	*Percentage*
Hepatitis B surface antigen	3	1.339
Hepatitis B surface antibody	18	8.072
Hepatitis B e antigen	2	1.005
Hepatitis B e antibody	8	3.980
Rapid plasma reagin	1	0.450

Out of the entire population eight patients were found to have positive hepatitis B surface antigen titers. This was an incidence of 1.37%. The cost of a hepatitis B surface antigen titer at the laboratory performing these tests is $24 per patient. In this population, therefore, the cost to detect each case was $1755.

In comparison, a positive rapid plasma reagin test result was found in six patients, for an incidence of 1.03%. One of these was felt to be a biologic false positive test result, due to intercurrent chickenpox. As the cost of a rapid plasma reagin test was only $10.50, the cost to detect each positive result was $1023.75. However, if only the true cases of syphilis are counted, the cost was $1228.50. According to standard protocol, the rapid plasma reagin test was also obtained at approximately 32 weeks' gestation and at delivery. As no new cases were detected at these times, the total cost for each case of syphilis detected reached $3685.50.

In the group that had a complete hepatitis profile, three positive hepatitis B surface antigen titers were found in 224 patients (see Table II). The incidence is thus very similar to that of the overall group, 1.34%. However, as a complete screen costs $80, the cost of detecting each case was $5973.33. Eighteen patients

were found to have hepatitis B surface antibody present, indicating past hepatitis infection and subsequent immunity. Thus 21 patients either had hepatitis B at that time or had been exposed to it in the past, for an incidence of 9.4%. The presence of the e antigen was also evaluated in this group. Predictably no patient who did not also have positive results for hepatitis B surface antigen showed the presence of e antigen. Of the three patients with positive results for hepatitis B surface antigen, two demonstrated e antigen. The third patient had antibody to the e antigen.

Of the eight cases of hepatitis B detected by routine screening tests, no patients had clinical jaundice or other symptoms suggestive of the diagnosis. One patient gave a history of previous diagnosis as a chronic carrier of hepatitis B. The other seven patients denied any history of hepatitis B exposure.

In the entire population of 585, nine patients gave a history of hepatitis. One of those, as mentioned above, was a chronic carrier. Four of the others stated that they had hepatitis B, while the remaining four were unsure. Six of these eight had complete serologic screening. Only two of these patients had serologic evidence of having had hepatitis B in the past. Interestingly, two of the patients with negative screens insisted that they had previously had hepatitis B.

Analysis of risk factors. Several of the known risk factors related to increased incidence of hepatitis B were also examined. None of the patients in the clinic admitted to intravenous drug abuse, so this risk factor could not be analyzed. Relatively few patients fell into each of the other risk categories. Eighteen patients had a history of blood transfusion, but none of the transfused patients were found to be infectious. Of the six who had hepatitis B testing done, one had hepatitis in the past as demonstrated by the presence of hepatitis B surface antibody. The incidence of hepatitis B in the transfused group was not significantly increased by χ^2 analysis.

Six patients had tattoos noted on physical examination. None of these had active hepatitis B; however, three of the six had serologic evidence of hepatitis B in the past. Of the six tattooed patients in the first group, one had active hepatitis. The increased incidence of past or present hepatitis B in the group 2 patients was statistically significant by χ^2 analysis, with a value of 11.98, $p < 0.01$. Analysis was not valid for group 1 because of the small incidence of both active hepatitis and tattoos.

Ten patients lived in institutional settings. None of them had active hepatitis; however, three of the patients had evidence of hepatitis B in the past. The χ^2 analysis showed this to be a highly significant difference, with a value of 15.4 ($p < 0.001$).

Patients who gave a history of either syphilis, gonvestigated. A total of 92 patients gave such historie Three of these patients had active hepatitis B, and fou had evidence of past infection. In the entire populatio with a past history of sexually transmitted diseases, th incidence of active hepatitis B was slightly increasec This increase was not statistically significant.

Comment

How serious a problem is hepatitis B in pregnancy A mother infected in the third trimester of pregnanc or who is a chronic carrier with a positive e antigen ha approximately a 90% chance of infecting her newbor infant.[3] Although transplacental infection does occu existing information appears to indicate that this is rare occurrence. It develops in about 5% of cases.[1] Most infections occur during birth or during the im mediate postpartum period. Although exposure to ma ternal blood and secretions is thought to be the rout of infection, cesarean section does not decrease the ris of infection.[4] Hepatitis B virus is also present in breas milk, although opinion is divided as to whether this i a significant route of infection.[1, 5]

Mothers who are chronic carriers have less of a ris of passing hepatitis B on to their infants compared wit those with acute hepatitis B. Variation in infectivity i related to the percentage of chronic carriers who hav e antigenemia. This varies from 10% to 71% of carrier but in general is higher in younger carriers, that is, th childbearing age group.[6, 7] If the mother has e antige nemia, the chance that her child will be infected is abou 90%; if she has e antibody, it is <10%.[6] Most of th infants infected at birth do not develop acute hepatitis Those rare infants that do so may die of fulminant live failure. Of the infants infected, 80% to 90% will becom chronic carriers.[2] Thus perinatal infection increases th pool of chronic carriers who will continue to expos others to hepatitis B during their entire lifetimes.

Although chronic carriers may have no obvious ill ness, they do not escape the effects of the hepatitis l virus. Studies indicate a higher incidence of chroni liver disease, including cirrhosis and liver failure, i hepatitis B carriers.[8] Some evidence also suggests tha they are at increased risk for hepatocellular carcinoma Thus these babies not only pose an immediate and con tinuing risk to others but may have an appreciabl shortened life span themselves.

Undetected cases of hepatitis B in prenatal patient also pose danger to hospital personnel. Because of th nature of labor and delivery, nurses and physicians ma have extensive exposure to blood and bodily secretion of a patient. Even roommates, who would not ordinaril be in danger of contracting hepatitis from a fellow pa tient, may be exposed via the shared bathroom in patient whose lochia is infectious.

Increasing concern for cost-effectiveness has mad

out documentation of their yield. This approach has its difficulties, however, in that a value cannot be assigned to human life. Hepatitis contracted in the neonatal period clearly can shorten life span, even if the infant does not experience an acute episode of hepatitis. What value can we place on the lives of these infants?

One approach is to compare routine hepatitis screening with an already accepted and widely used screening test. If we are willing to spend nearly $4000 to detect each case of syphilis, perhaps less than $2000 is reasonable for each hepatitis case detected.

It must be noted that other methods of disease detection would be relatively ineffective in diagnosing these cases of hepatitis. Traditionally, the history and physical examination are the basic screening tests for underlying diseases. None of the eight patients with positive hepatitis test results in this study would have been detected by physical examination and only one by history. The lack of specificity of history is additionally demonstrated by the finding that only one of nine patients giving a history of hepatitis was infectious. Nor can the lack of symptoms in these patients be attributed to chance; previous studies have shown that up to 60% of cases of hepatitis B are asymptomatic.[9] This is confirmed in the present study as well. Six patients gave a history of hepatitis, and only two of these had evidence of having had hepatitis B, for an incidence of 0.89%. Serologic testing of the general population, however, demonstrated a true incidence of 9.4%. This would indicate that only 10% of the patients who had hepatitis B were previously diagnosed as such.

Factors that are known to increase the risk of hepatitis B infection include intravenous drug abuse, tattoos, and blood transfusion.[9] In addition, because of the possibility of sexual transmission, patients with a history of other sexually transmitted diseases may be at increased risk.[10] Institutionalized people, whether in prison or in homes for the retarded, also have a greater risk of hepatitis B.[11] One possible approach to the problem of detecting the most cases of hepatitis B at the lowest cost would be to target these groups for more intensive screening. In this sample, selecting subgroups at higher risk to test also would have been ineffective. Part of this failure was due to the small number of positive cases, which made statistical analysis impossible in some of the subgroups. However, even with these small numbers, the presence of tattoos and institutional living were clearly linked with exposure to hepatitis. Patients with a history of sexually transmitted diseases also had increased risk. Identification of these subgroups did not help to detect infectious patients and thus detect infants at risk. The patients with the risk factors were more likely to have had hepatitis B in the past rather than to have active hepatitis. The fact that risks did not seem to be increased in the population that had received transfusions may be due to the young age of the population. They would have been transfused after widespread screening of blood donors for hepatitis B was initiated in this country, markedly reducing the risk for contracting this type of hepatitis from banked blood.

Screening for hepatitis B in this entire population therefore would seem to be the only way to detect the majority of active cases during pregnancy. While the full hepatitis screen performed in the second group in our study gave added insight into the problem in a study situation, it is clearly not necessary in clinical practice. A single hepatitis B surface antigen value, preferably obtained in the third trimester, would seem to be of value in the management of patients of this population. A test done at this time had less chance of missing a case of hepatitis contracted late in pregnancy, and if a positive test result for hepatitis B surface antigen is found it is likely to persist to term. In cases of positive results, further screening may be done to clarify e antigen status and attempt to differentiate chronic carriers from those having acute episodes of hepatitis.

This recommendation can be generalized to include similar patient populations: inner-city, adolescent, predominantly black, low socioeconomic class. Generalization to populations of different socioeconomic class or living situation cannot be justified at this time. Investigation of these populations might be indicated in the future to further define this problem.

REFERENCES

1. Lee AKY, Ip HMH, Wong VCW. Mechanisms of maternal-fetal transmission of hepatitis B virus. J Infect Dis 1978;138:668-71.
2. Stevens CE, Toy PT, Tong MJ, et al. Perinatal hepatitis B virus transmissions in the United States: prevention by passive-active immunization. JAMA 1985;253:1740-5.
3. Beasley RP, Hwang LY. Postnatal infectivity of hepatitis B surface antigen-carrier mothers. J Infect Dis 1983;147: 185-90.
4. Stevens CE. Viral hepatitis in pregnancy: the obstetrician's role. Clin Obstet Gynecol 1982;25:577-83.
5. Steven MM. Pregnancy and liver disease. Gut 1981;22: 592-614.
6. Szmuness W, Neurath AR, Stevens CE, Strick N, Harley EJ. Prevalence of hepatitis B "e" antigen and its antibody in various HBsAg carrier populations. Am J Epidemiol 1981;113:113-20.
7. Aldershvile J, Skinhij P, Frosner GG, et al. The expression pattern of hepatitis B e antigen and antibody in different ethnic and clinical groups of hepatitis B surface antigen carriers. J Infect Dis 1980;142:18-22.
8. Shiraki K, Yoshihara N, Kawana T, Yasui H, Sakurai M. Hepatitis B surface antigen and chronic hepatitis in infants born to asymptomatic carrier mothers. Am J Dis Child 1977;131:644-7.
9. Clarke DD, Kao HW. Meaningful markers for hepatitis Dx and Rx. Contemp Ob Gyn 1983;21:31-50.
10. Minkoff H. Hepatitis. Clin Obstet Gynecol 1983;26: 178-85.
11. Seeff LB, Koff RS. Passive and active immunoprophylaxis of hepatitis B. Gastroenterology 1984;96:958-78.

Cardiac output response to prostaglandin E_2–induced abortion in the second trimester

Donald C. Willis, M.D., Donald Caton, M.D., J. Patrick Levelle, M.D., and Tina Banner, R.N.

Gainesville, Florida

Maternal cardiac output changes were evaluated in 10 patients undergoing second-trimester abortion induced by prostaglandin E_2 suppositories. The peak cardiac output increased by an average of 64.5% above preinduction values. This is similar to the percent increase described during oxytocin-induced labor at term. Inasmuch as resting cardiac output tends to be higher in midpregnancy than at term, absolute values during prostaglandin E_2 induction were higher than those observed in laboring term patients. (Am J Obstet Gynecol 1987;156:170-3.)

Key words: Prostaglandin E_2, prostaglandin, cardiac output, elective abortion, labor, pregnancy, cardiac

Prostaglandin E_2 from vaginal suppositories, effective for use in second-trimester abortion, is known to be absorbed systemically.[1] Whether the absorbed drug induces cardiovascular effects in pregnancy is not known but is a pertinent question in view of the potential use in patients with cardiovascular disease. A relative contraindication for the use of prostaglandin suppositories in pregnancy includes women with hypertension or known cardiovascular disease.[2, 3]

Hemodynamically, the most significant cardiovascular alteration observed during pregnancy is the increase in cardiac output. In the past, cardiac output determinations in otherwise healthy pregnant women have been limited by the invasiveness of the procedure. Recently, Doppler determinations of cardiac output have been shown to correlate with the thermal dilution method in nonpregnant subjects ($r = 0.94$, 0.97, and 0.94).[4-6] The present study uses this noninvasive Doppler technique to measure cardiac output response in prostaglandin E_2–induced abortions.

Material and methods

Ten women treated with prostaglandin E_2 vaginal suppositories (Prostin E_2, Upjohn) for midtrimester abortion were included in this investigation. The study was approved by the Institutional Review Board, and informed consent was required for inclusion. Gestational age ranged from 15 to 23 weeks. Three of the 10 abortions were for fetal death, and the others were for medical indications. Patient profiles are listed in Table I.

Three of 13 patients were excluded from the study. Patient No. 6 (Table I) was taking digoxin for Wolff-Parkinson-White syndrome. Patient No. 8 requested continuous epidural analgesia for relief of pain. This form of analgesia has been shown to limit the increase in cardiac output observed in laboring women at term.[7] Patient No. 9 was known to be hypertensive and was treated with methyldopa before induction; she required intravenous hydralazine for blood pressure control during the abortion. Since hydralazine can increase cardiac output, this patient was excluded from the study.[8]

Cardiac output was determined by an ultrasonic Doppler computer (Ultracom, Lawrence Medical Systems, Inc.), a technique that calculates cardiac output by multiplying measurements of aortic blood velocity by aortic diameter. The smallest diameter of the ascending aorta was measured by A-mode echocardiography. Ascending aortic blood velocity was determined by continuous-wave Doppler. Since the aortic root diameter does not change significantly between systole and diastole, cardiac output was then calculated by the Ultracom instrument with use of systolic velocity, cross-section area of the aorta (computed from aortic diameter), and heart rate. Each cardiac output was obtained by taking the average of at least three separate measurements obtained within a 2-minute period. Baseline measurements were obtained before induction and approximately every 90 minutes thereafter. All cardiac output determinations were performed by one of us.

Cardiac output was measured with patients in a 15-degree semirecumbent position because the left lateral position makes Doppler measurements technically

From the Departments of Anesthesiology and Obstetrics and Gynecology, University of Florida College of Medicine.
Received for publication January 27, 1986; revised August 12, 1986; accepted August 19, 1986.
Reprint requests: Donald C. Willis, M.D., Department of Obstetrics and Gynecology, University of Hawaii, 1319 Punahou St., Honolulu, HI 96826.

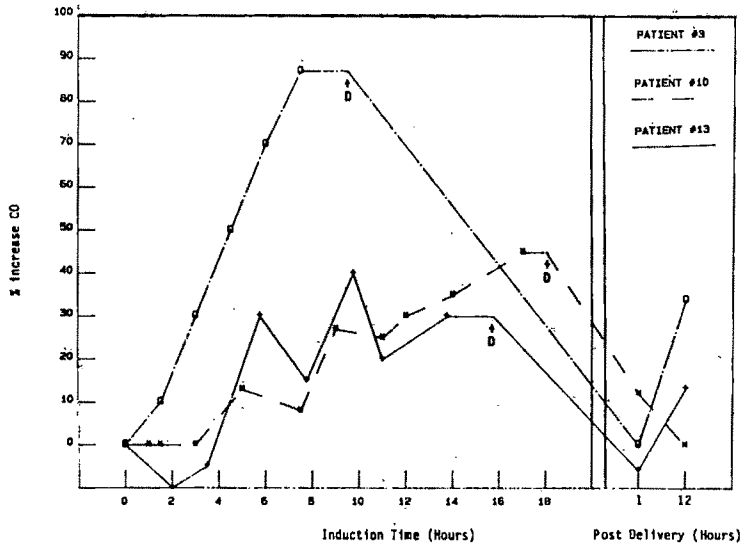


Fig. 1. Representative cardiac output response during prostaglandin E_2–induced abortion.

Table I. Cardiac output during prostaglandin E_2–induced abortion

Patient No.	*Cardiac output maximal response*		*Duration of induction (hr/min)*	*Total prostaglandin E_2 doses*	*Gestation (wk)*	*Parity*	*Preinduction fetal status*
	L/min	*% increase*					
1	21.6	116	16:00	5	21	1	Alive
2	14.5	75	8:20	2	22	3	Dead
3	20.4	88	9:25	4	23	0	Alive
4	5.3	26	7:45	2	22	2	Dead
5	21	93	16:40	5	22	0	Alive
6*	5.8	21	16:05	4	16	3	Dead
7	12.1	73	11:40	3	17	0	Alive
8†	9.2	48	8:05	2	22	3	Alive
9‡	13.6	89	12:00	3	20	2	Dead
10	8.6	46	18:00	5	17	0	Alive
11	8.6	46	12:30	3	23	2	Alive
12	11.7	41	13:25	4	20	1	Alive
13§	9.0	41	16:40	5	15	0	Dead

*Wolff-Parkinson-White syndrome; patient excluded from study.
†Continuous epidural analgesia; excluded from study.
‡Chronic hypertension; excluded from study.
§Fetal death occurred 3 to 4 weeks before abortion.

more difficult to perform. A change in body position during midpregnancy (20 to 24 weeks) causes only a small change in cardiac output (8%),[9] so measurements with the patient semirecumbent were considered reliable.

In midpregnancy, prostaglandin administration is associated more with an increase in uterine tone than with more frequent rhythmic contractions.[10] It was therefore difficult to time cardiac output determinations with uterine contractions. When it was clinically apparent, cardiac output was measured between contractions.

Prostaglandin E_2 vaginal suppositories, 20 mg, were inserted every 3 to 4 hours. Analgesia was provided with a combination of promethazine, 25 mg, and either intravenous meperidine hydrochloride, 25 to 50 mg, or butorphanol (Stadol, Bristol Laboratories), 1-2 mg. Patient No. 8, not included in this study (Table I), received continuous epidural analgesia.

Data were analyzed by use of paired or unpaired *t* test and by calculating Pearson correlation coefficients with the Systat statistical program (Systat, Inc.) on a Dec Rainbow computer.

Results

In this investigation, cardiac output increased in every patient during prostaglandin E_2 induction. The average preinduction cardiac output was 7.8 ± 2.2 L /min (mean ± SD). These values did not differ statistically from cardiac output measured with the same

Table II. Hemodynamic values during prostaglandin E_2–induced second-trimester abortion

	Mean ± SD	*Range*
Cardiac output (L/min)		
Preinduction	7.7 ± 2.2	4.2-10.9
Maximal response	13.3 ± 5.6*	5.3-21.6
Cardiac index (L/min)/m^2		
Preinduction	4.4 ± 1.2	2.5-6.5
Maximal response	7.5 ± 2.9*	3.1-12.2
Stroke volume (ml)		
Preinduction	96.8 ± 30.8	53-148
At maximal cardiac output	119.1 ± 43.7*	60-189
Pulse rate (bpm)		
Preinduction	82.3 ± 11.5	56-99
At maximal cardiac output	117.6 ± 15.6*	98-156
Blood pressure (mm Hg)		
Preinduction		
Systolic	108 ± 12.5	92-130
Diastolic	67.5 ± 5.9	58-78
At maximal cardiac output		
Systolic	111.1 ± 18.9	96-149
Diastolic	62.8 ± 9.6	50-80

*P < 0.05 compared with preinduction values.

technique, as part of another study, in 17 women between 17 to 23 weeks' gestation ($p > 0.01$).[11]

The maximum cardiac output achieved during induction was 13.3 ± 5.6 L/min. The average increase in cardiac output was 5.5 L/min, or 64.5% above preinduction values. There was considerable variation among patients. The increase in cardiac output was accompanied by an increase in both heart rate and stroke volume. Preinduction cardiac output, cardiac index, heart rate, and stroke volume increased significantly during treatment ($p < 0.01$) (Table II).

The time that peak cardiac output is sustained is important. However, this is difficult to describe or quantitate because of the variable length for inductions. Generally, cardiac output increased soon after prostaglandin E_2 induction began. Cardiac output continued to increase during induction with peak values obtained just before delivery. Representative cardiac output responses are depicted in Fig. 1.

The change in cardiac output was not related to length of gestation, parity, duration of induction, or total dose of prostaglandin E_2 (Table I). However, patients with higher preinduction cardiac output showed a significantly greater increase during induction ($r = 0.684$, $p < 0.05$). The mean cardiac output was not statistically different in cases of abortion for fetal death in utero compared to those for other indications.

No cardiovascular morbidity occurred among patients in this study.

Comment

The percent increase in cardiac output found in this study is similar to that described for labor at term.[12] However, the absolute cardiac output in L/min is greater because cardiac output is higher during midpregnancy than at term.[3] Cardiac output has been reported to rise 2.5 L/min during the second stage of labor at term.[7] In the present study the cardiac output increased by an average of 5.5 L/min. The magnitude of cardiac output increase during prostaglandin E_2 termination would appear to be of clinical significance in patients with known cardiovascular disease or limited cardiac reserve.

In the classic studies on cardiac output of Uelanc and Hansen[13] which used a dye dilution technique, they reported a range of 3.3 to 13.0 L/min/hr for women at term before induction of labor. Our preinduction values showed less range (4.2 to 10.9 L/min/hr). However, during active labor we did see a considerable variation among patients. This could reflect individual patient response to labor, prostaglandin, hormonal milieu associated with fetal death, administered analgesics and/or response to pain.

Narcotic analgesics and sedatives have no consistent affect on cardiac output.[7] During labor, response to pain can increase cardiac output.[12] However, it would be unethical to perform an abortion without providing adequate analgesics consistent with each patient's request. We feel that the analgesics used reflect the clinical situation under which abortion is performed.

It is beyond the scope of this study to determine whether prostaglandin E_2 has a direct effect on the maternal cardiovascular system contributing to the significant increase in cardiac output observed. There is some information that suggests this possibility, since in nonpregnant individuals, prostaglandin E_2 is a known potent vasodilator resulting in a lowering of blood pressure, increase in heart rate, and cardiac output.[14]

Although the mean cardiac output increase was less among patients undergoing pregnancy termination for fetal death in utero (3.6 ± 2.6 L/min) versus viable fetus (6.0 ± 3.6 L/min), statistical significance was not reached ($p = 0.2376$). This may reflect the small number of patients with intrauterine fetal death and the wide range of cardiac output response. Further study would be needed to resolve this issue.

Whether other methods used to induce labor for second-trimester abortion cause similar increases in cardiac output is unknown. We studied one patient, not included in this investigation, who underwent abortion by use of intra-amniotic urea. Cardiac output increased 2.8 L/min, or 50% above preinduction values.

With patients known or suspected to have cardiovascular disease, the increase of cardiac output that occurs during induction of labor for second-trimester abortion should be considered. Surgical dilatation and evacuation could be considered, since this method may impose less cardiovascular alterations.

REFERENCES

1. Sandberg F, Ingelman-Sundberg A, Ryden G, et al. The absorption of tritium-labelled prostaglandin E_1 from the vagina of non-pregnant women. Acta Obstet Gynecol Scand 1968;47:22.
2. The Upjohn Company. Prostin E2 vaginal suppository (dinoprostone) [Package insert]. Kalamazoo, Michigan: Upjohn, 1981.
3. Patterson SP, White JH, Reaves EM. A maternal death associated with prostaglandin E_2. Obstet Gynecol 1979; 54:123.
4. Huntsman LL, Stewart DK, Barnes SR, et al. Noninvasive Doppler determination of cardiac output in man: clinical validation. Circulation 1983;67:593.
5. Chandraratna PA, Nanna M, McKay C, et al. Determination of cardiac output by transcutaneous continuous-wave ultrasonic Doppler computer. Am J Cardiol 1984; 53:234.
6. Gallagher IJ, Banner MJ, Cavallaro DL. Cardiac output measured noninvasively by continuous wave ultrasonic Doppler computer. Anesth Analg 1983;62:245.
7. Ueland K, Hansen JM. Maternal cardiovascular dynamics. III. Labor and delivery under local and caudalanalgesia. Am J Obstet Gynecol 1969;103:8.
8. Gillman AG, Goodman LS, Rall TW, Murad F, ed. Goodman and Gillman's the pharmacological basis of therapeutics, 7th ed. New York: Macmillan, 1985.
9. Ueland K, Novy MJ, Peterson EN, et al. Maternal cardiovascular dynamics. IV. The influence of gestational age on the maternal cardiovascular response to posture and exercise. Am J Obstet Gynecol 1969;104:856.
10. Wigvist N, Bygdeman M, Kwon Su, et al. Effect of prostaglandin E_1 on the midpregnant human uterus: intravenous, intramuscular, intra-amniotic, and vaginal administration. Am J Obstet Gynecol 1968;102:327.
11. James CF, Banner T, Levelle P, Caton D. Noninvasive determination of cardiac output throughout pregnancy [Abstract]. Washington DC: Society of Obstetric Anesthesia and Perinatology, seventeenth annual meeting, May 9, 1985.
12. Metcalfe J, Ueland K. Maternal cardiovascular adjustments to pregnancy. Prog Cardiovasc Dis 1974;16:363.
13. Ueland K, Hansen JM. Maternal cardiovascular dynamics. II. Posture and uterine contractions. Am J Obstet Gynecol 1969;103:1.
14. Patel N. Prostaglandins: newest of the hormones? Ann Intern Med 1970;73:483.

Prostaglandin E_2 release on the fetal and maternal sides of the amnion and chorion-decidua before and after term labor

John A. McCoshen, Ph.D., Kimberly A. Johnson, B.Sc., Norman H. Dubin, Ph.D., and Ramesh B. Ghodgaonkar, B.Pharm.

Winnipeg, Manitoba, Canada, and Baltimore, Maryland

Release of prostaglandin E_2 on each of the fetal and maternal sides of the fetal membranes (8 cm^2) from term cesarean (no labor) and spontaneous vaginal (labor) deliveries was studied with the use of dual-compartment perfusion chambers. Postlabor amnion released significantly ($p < 0.05$) more total prostaglandin E_2 (5.66 ± 1.02 ng, mean ± SEM) than prelabor tissue (3.34 ± 1.76 ng) with equivalent prostaglandin E_2 levels being released on both sides. A net decrease ($p < 0.001$) in prostaglandin E_2 release by chorion-decidua was identified after labor (fetal = 0.12 ± 0.05 ng; maternal = 0.17 ± 0.09 ng) when compared with that before labor (fetal = 1.28 ± 0.69 ng; maternal = 2.31 ± 0.56 ng). Amnion-chorion-decidua released more prostaglandin E_2 on the fetal side after labor (2.37 ± 1.43 ng) than prior to labor (1.49 ± 0.60 ng); however, prostaglandin E_2 on the maternal side was significantly less ($p < 0.05$) after labor (0.24 ± 0.12 ng) when compared with that of the prelabor membrane (2.03 ± 1.08 ng). Elution of prostaglandin E_2 from preincubated membranes anc endogenous membrane prostaglandin E_2 content showed similar results. Thus concentrations of prostaglandin E_2 on the maternal side of the fetal membranes appear diminished after spontaneous labor despite an increased release from the fetal surface. (Am J Obstet Gynecol 1987;156:173-8.)

Key words: Prostaglandin E_2, fetal membrane sides, labor

From the Department of Obstetrics, Gynecology and Reproductive Sciences, University of Manitoba Faculty of Medicine, and the Department of Gynecology and Obstetrics, The Johns Hopkins University.

Supported by the Medical Research Council of Canada and the Manitoba Medical Services Foundation, Inc.

Received for publication December 2, 1985; revised April 16, 1986; accepted August 12, 1986.

Reprint requests: Dr. J. A. McCoshen, Department of Obstetrics, Gynecology and Reproductive Sciences, University of Manitoba, 59 Emily St., Winnipeg, Manitoba, Canada R3E 0W3.

Production of prostaglandins by fetal membranes, including amnion and chorion, as well as accumulated prostaglandins in the amniotic fluid may be associated with the initiation and/or maintenance of human labor. Cervical dilatation and induction of labor occur after cervical exposure to or infusion of amniotic fluid with prostaglandin E_2 (PGE_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$).[1, 2] Proposed sources of amniotic fluid prostaglandins include the fetal membranes and fetal kidney since PGE_2 and $PGF_{2\alpha}$

Table I. Release of PGE_2 by term fetal membranes

Membrane type*	Net PGE_2 (ng) (mean ± SEM)	
	No labor (cesarean section)	*Labor (vaginal delivery)*
Amnion	3.49 ± 0.81	7.04 ± 1.91
Chorion-decidua	2.00 ± 0.69	0.76 ± 0.27
Amnion-chorion with decidua	2.38 ± 0.45	1.75 ± 0.53
Amnion/chorion-decidua†	1.85 ± 0.50	—

Mean ± SEM net PGE_2 released by term fetal membranes of standardized surface area of 8 cm^2 into 18 ml of medium during 2 hours of preincubation at 20° C.

*n = 8 for each value.

†Amnion separated from chorion-decidua and then replaced.

production by amniotic cells and concentrations in fetal urine increase significantly between the onset of labor and parturition.[3-6] Furthermore, both placenta and decidualized endometrium are production sites of prostaglandins.[7,8] The findings of increased fetal membrane production and amniotic fluid concentrations of prostaglandins have led to speculation that the availability of prostanoid hormones to the myometrium increases about the time of onset of labor.[9] Thus the amount of prostaglandin released on the maternal side of the gestational sac would necessarily increase with the onset of labor.

The present study tested the hypothesis that during the course of labor PGE_2 release by fetal membranes favors an increased availability of this prostaglandin to the myometrium. Furthermore, the effect of membrane dissociation of amnion from chorion-decidua on the release of PGE_2 was also tested since alterations in membrane transport and physiologic properties occur under those conditions.[10] To facilitate this study PGE_2 release was equated to a constant surface area of fetal membrane maintained in dual-compartment perfusion chamber systems designed to simulate in vivo conditions.

Material and methods

Reflected fetal membranes consisting of amnion, chorion-decidua, amnion-chorion with adherent decidua, and amnion mechanically separated from chorion-decidua and subsequently replaced in contact were used in these experiments. The membranes were obtained from four normal repeat term cesarean sections, without labor, between 38 and 39 weeks' gestation, and from four normal pregnancies delivered vaginally after spontaneous labor and parturition between 39 and 41 weeks' gestation. From each afterbirth the reflected membranes were cut free distal to the placenta and washed in cold phosphate-buffered saline, at pH 7.4, to remove residual blood. Disks (8 cm^2) of either amnion, chorion-decidua, amnion-chorion with adherent decidua, or, in the case of cesarean deliveries, amnion separated from chorion-decidua and then replaced were cut and mounted onto circular 22-mesh stainless steel screens as described previously.[10] Respective membrane types from each pregnancy were prepared in duplicate and mounted in dual-compartment perfusion chambers, such that each hemichamber enclosed either the fetal or the maternal membrane side. The membranes were checked for leakage first by addition of 9 ml of synthetic amniotic fluid at pH 7.4 to the fetal chamber and observation of the maternal chamber for 10 minutes. The synthetic amniotic fluid was used as an incubation medium because of its superior qualities in maintaining membrane viability and metabolism in vitro.[11] After confirmation of a leak-free preparation, the maternal chamber was filled to 9 ml with synthetic amniotic fluid. The membranes were preincubated for 2 hours at room temperature (20° C) with a change of synthetic amniotic fluid at 1 and 2 hours, respectively. After each time period, a 0.5 ml sample of synthetic amniotic fluid was removed from each hemichamber, pooled, and quick-frozen in acetone–dry ice (−70° C). After preincubation the chambers were placed in a water bath at 37° C and the temperature of the perfusion chambers was allowed to equilibrate for 15 minutes.

After equilibration a 0.5 ml aliquot of fluid was removed from each hemichamber at 0 hours and again at 2 and 4 hours. During incubation, chamber fluids were aerated with a continuous circulation of humidified filtered air delivered via a 22-gauge needle. After sampling, the chamber fluids were replenished with synthetic amniotic fluid to maintain a constant volume of 9 ml on each side.

At the end of each experiment all membranes were blotted, weighed, and fixed in neutral buffered formalin for histologic evaluation of viability. Paraffin sections were stained with 1% toluidine blue.

Reflected samples of chorion-decidua from an additional five cesarean deliveries and five vaginal deliveries were each homogenized in 1:1 (w/v) synthetic amniotic fluid, pooled, and centrifuged at 5000 × g for 30 minutes at 4° C. The supernatants were collected and again centrifuged for 30 minutes. Supernatants were then diluted 1:8 in synthetic amniotic fluid and evaluated for PGE_2 content in each of the cesarean and vaginal chorion-decidua pools.

Radioimmunoassay of PGE_2 was performed according to the method of Dubin et al.[12] with the use of rabbit anti-PGE_2 antibody produced by antigen coupling to bovine serum albumin. Samples of synthetic amniotic fluid containing antisera were incubated overnight at

Table II. Results of statistical analysis

Type of membrane	Mode of delivery		Membrane side			
			No labor (cesarean section)		Labor (vaginal delivery)	
	F value*	p†	F value	p	F value	p
Amnion	4.29	<0.05	0.18	NS	0.24	NS
Chorion-decidua	37.91	<0.001	1.92	NS	0.30	NS
Amnion-chorion with decidua	2.64	NS	0.28	NS	4.22	<0.05
Amnion/chorion-decidua‡	—	—	9.02	<0.01	—	—

Summary of multiple analysis of variance of term fetal membrane PGE_2 released during a period of time according to mode of delivery (cesarean section without labor versus spontaneous labor and vaginal delivery) and membrane side (fetal versus maternal).

*F Values at 1 to 48 degrees of freedom.

†Probability: NS = Not significant.

‡Amnion separated from chorion-decidua and then replaced.

4° C along with the appropriate tracer. Separation of bound from free antigen was accomplished with dextran-coated charcoal. The limit of detection of PGE_2 was 12 pg/ml with intra-assay and interassay coefficients of variation of <10% and 15%, respectively. PGE_2 values from membrane incubates were converted to total PGE_2 released on each of the fetal and maternal sides; that is, radioimmunoassay PGE_2 values in picograms per milliliter × 9 ml of medium on each side equals PGE_2 released by 8 cm^2 of membrane into the fetal or maternal hemichamber. Thus the results reflect total PGE_2 release by a standard membrane surface area common to each experiment. All radioimmunoassay results were analyzed by multiple analysis of variance to test the treatment effects of type of membrane, membrane side, mode of delivery, and incubation time. Multiple two-tailed *t* tests and analysis of correlation were also used for testing specific variables.

Results

During preincubation at 20° C, PGE_2 eluted from all membrane types (Table I) with the highest levels originating from amnion obtained from vaginal deliveries. The lowest concentrations of PGE_2 were eluted from chorion-decidua from vaginal deliveries. The profiles of PGE_2 release in nanograms per hemichamber during 4 hours of incubation at 37° C for each membrane type from the two modes of delivery are presented in Figs. 1 to 4. Statistical analysis identified significant differences in PGE_2 release by the various membrane types, according to side and mode of delivery (Table II). In the case of amnion, significantly higher PGE_2 levels were released during a period of time by vaginal membranes than by cesarean membranes (Fig. 1) whereas chorion-decidua obtained from vaginal deliveries failed to produce a net increase in PGE_2 during a period of time when compared with membranes obtained from cesarean sections (Fig. 2). The amnion-chorion with adherent decidua from both cesarean and vaginal deliveries released substantial PGE_2 (Fig. 3); however, significant differences in total PGE_2 were identified relative to the membrane side only. Membranes from cesarean deliveries consisting of dissociated amnion and chorion-decidua produced a net accumulation of PGE_2 during a period of time but the concentration profiles relative to side differed significantly from those of intact amnion-chorion with adherent decidua also derived from cesarean deliveries (Fig. 4). Thus mechanical disruption of cellular contact between amnion and chorion-decidua obtained from cesarean deliveries resulted in a reduction of PGE_2 release into the maternal hemichamber. PGE_2 on the fetal or amniotic side, however, remained equivalent to that of the fetal sides of both isolated amnion membranes and amnion-chorion with adherent decidua membranes from cesarean deliveries. Histologic assessment of each type of membrane failed to identify changes in membrane viability subsequent to incubation when compared with sections of fresh specimens. Pooled extracts of chorion-decidua from cesarean deliveries contained the equivalent of 6.5 ng of PGE_2 per gram of tissue whereas endogenous PGE_2 from chorion-decidua obtained after spontaneous labor and vaginal delivery was almost fourfold less, at 1.8 ng/gm of tissue.

Comment

A net increase in prostaglandin production by amniotic cells derived from term pregnancy without labor compared with pregnancy terminating with spontaneous labor and parturition has been described previously.[7, 8, 13] Studies on the production of various prostaglandins by chorionic and decidual tissues, in addition to amnion, have led to speculation that the fetal membranes may be involved in the initiation and maintenance of labor.[8, 13] Furthermore, intra-amniotic injection of prostaglandins is known to induce labor in a

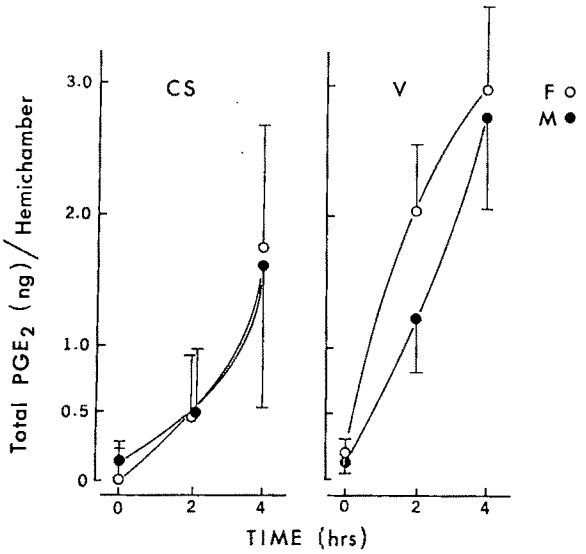


Fig. 1. PGE_2 release during 4 hours by 8 cm^2 disks of amnion derived from cesarean *(CS)* and vaginal *(V)* deliveries. Total PGE_2 release by *V* amnion was significantly greater than that by *CS* amnion; however, no significant difference between fetal *(F)* and maternal *(M)* PGE_2 concentrations exists within each of *CS* or *V* delivered membranes.

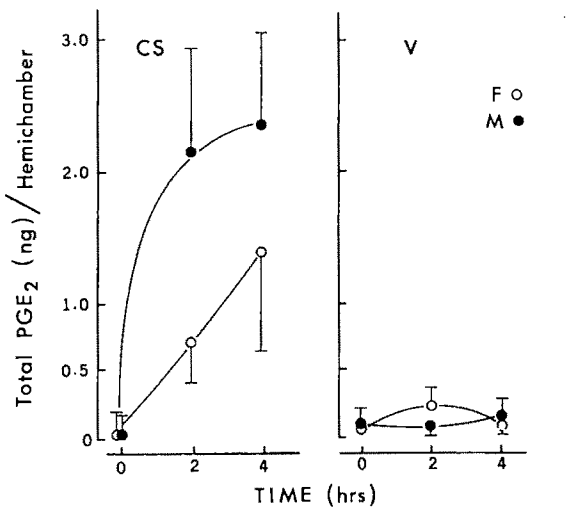


Fig. 2. Profiles of PGE_2 release during 4 hours by chorion-decidua of 8 cm^2 surface area from cesarean section *(CS)* and vaginal *(V)* deliveries. A significant difference ($p < 0.001$) exists in total PGE_2 release by *CS* versus *V* delivered membranes that is associated with decreased PGE_2 concentrations on the fetal *(F)* and maternal *(M)* sides of *V* delivered membranes.

number of circumstances.[2] Presumably, prostaglandin injection simulates the condition of increased amniotic fluid prostaglandin concentrations associated with term gestation.

The present study suggests that the known increase in amniotic fluid PGE_2 concentration occurring between the onset of labor and parturition may result in large part from increased amniotic productivity of

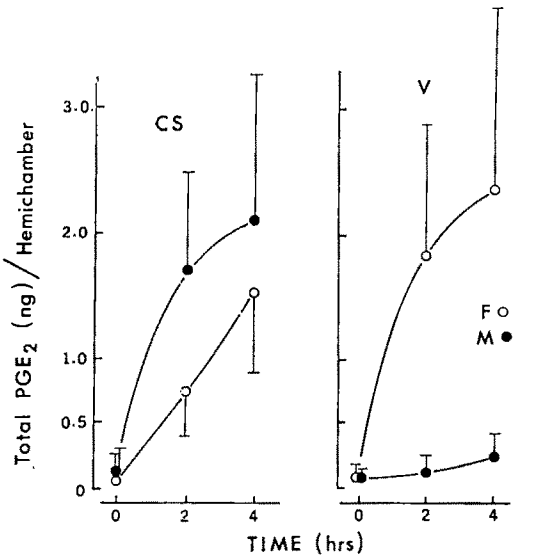


Fig. 3. PGE_2 release during 4 hours by composite 8 cm^2 disk of intact membranes consisting of amnion retained in cellula contact with chorion-decidua. PGE_2 increases on the fetal *(F* side of vaginal *(V)* delivered membranes were similar to thos of amnion, whereas PGE_2 on the maternal *(M)* side declinec to levels similar to those of chorion-decidua membranes fron cesarean section *(CS)* deliveries.

PGE_2 during that time. The finding of equivalent con centrations of PGE_2 on fetal and maternal sides of th amnion implies that amniotic PGE_2 becomes accessibl to the underlying chorion and possibly available fo transport by chorion-decidua to the myometrium However, the data on directional output of PGE_2 afte vaginal delivery by intact amnion-chorion with adher ent decidua, while reaffirming an increase in PGE_2 or the amniotic or fetal side, clearly display a substantia decline in net PGE_2 available to the maternal sid of the membranes. It is possible that the chorionic decidual component of amnion-chorion with adheren decidua from vaginal delivery possesses either a lo capacity for PGE_2 production and/or release or an al teration in ability to rapidly metabolize amniotic PGE_2 during transport. Chorion-decidua from vaginal deliv ery displayed almost no net PGE_2 release during a pe riod of time, unlike chorion-decidua from cesarean de liveries. Whatever functional mechanism(s) exists to ac count for this observation, it is apparent that unde these experimental conditions the availability of PGE_2 to the maternal or myometrial side is substantially re duced after spontaneous labor and delivery. Thus these results are contrary to the concept that PGE_2 produc tion by reflected fetal membranes avails increasing con centrations of the prostaglandin to the myometrium Additional evidence in support of this view was iden tified where endogenous PGE_2 content of chorion decidua from vaginal deliveries was almost fourfold les than that of tissue from cesarean delivery, suggestin

a net decline in PGE_2 on the maternal side of the membranes sometime during labor. Similarily, elution of PGE_2 from chorion-decidua during preincubation was approximately threefold less by vaginal delivery tissue than by cesarean delivery tissue.

It is possible that these results reflect a short-term decline in membrane PGE_2 production or the availability of membrane prostaglandin precursors, such as arachidonic acid,[13, 14] subsequent to or during parturition. However, this may not be the case since amniotic PGE_2 release increased at a time coincident to a decrease in chorionic-decidual PGE_2 concentrations. Also composite amnion-chorion with adherent decidua membranes from vaginal deliveries display differential PGE_2 release similar to that on the fetal and maternal sides of isolated amnion and chorion-decidua, respectively. Alternatively, the decidual component of chorion-decidua may rapidly convert PGE_2, produced by amnion and chorion, to $PGF_{2\alpha}$. Therefore, PGE_2 levels on the fetal side of isolated chorion-decidua would be expected to remain high, but this was not the case.

In those experiments where amnion and chorion-decidua were separated and subsequently replaced in contact, PGE_2 release by amnion without chorion-decidua from cesarean deliveries more closely resembled that of intact amnion-chorion with adherent decidua from membranes delivered vaginally than intact amnion-chorion with adherent decidua from cesarean deliveries. This unusual finding may suggest that the trauma of labor influences chorionic-decidual PGE_2 release or that an interaction between the fetal membranes exists relative to the regulation of PGE_2 production. Alterations in fetal membrane transport properties have been observed under similar experimental conditions in that decidual prolactin transport ceases after dissociation of amnion from chorion-decidua.[10]

In summary, the hypothesis of this study, that the availability of fetal membrane PGE_2 to the maternal or myometrial surface increases from a time before the onset of labor until parturition, must be rejected. The results suggest that endogenous chorionic-decidual content and release of PGE_2 decrease significantly sometime during labor. The possibility that this change is acute, occurring during parturition, cannot be excluded; however, amniotic PGE_2 release was substantially increased after parturition. Thus the availability of chorionic-decidual PGE_2 to the myometrium may indeed decline during labor, suggesting that differential metabolic changes occur in the fetal membranes during this time. Possibly chorionic-decidual PGE_2 is rapidly converted to some other metabolite. Finally, membrane trauma resulting from labor may account in part for the alteration in chorionic-decidual PGE_2 release since spontaneous membrane rupture and amniotomy,[15] amniotic sac distention,[16] and chorioamnionitis[17] are associated with the initiation of labor.

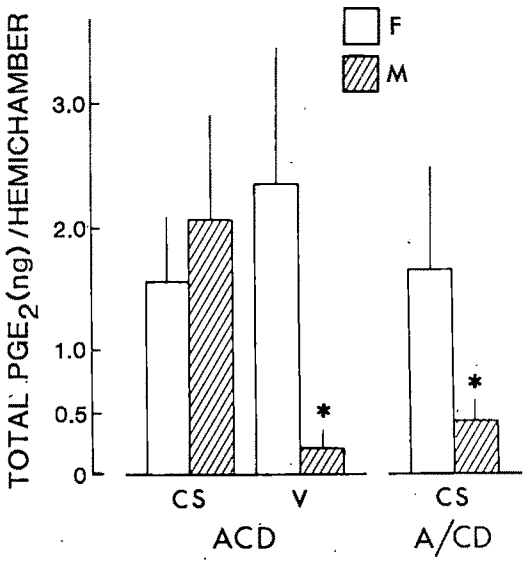


Fig. 4. Comparison of net 4 hour release of PGE_2 by composite 8 cm^2 disks of amnion-chorion membranes with adherent decidua *(ACD)* from cesarean section *(CS)* and vaginal *(V)* deliveries and dissociated disks *(A/CD)* of membranes from *CS* deliveries. Although *A/CD* membranes came from cesarean sections, PGE_2 production was more similar to that of *ACD* from *V* deliveries than from *CS* deliveries. A significant difference exists in PGE_2 concentrations on the maternal *(M)* side versus the fetal *(F)* side of *ACD* from *V* deliveries ($p < 0.05$) and *A/CD* from *CS* deliveries ($p < 0.01$).

Thus, considering the numerous variables involved in the physiology of labor, membrane trauma may warrant further attention for study. However, some other event(s) is then required to initiate the process of labor, leaving the uterus, placenta, and fetus as possible sites of pertinent activity.

REFERENCES

1. Wingerup L, Andersson K-E, Ulmsten U. Ripening of the uterine cervix and induction of labor with prostaglandin E2 in viscous gel. Acta Obstet Gynecol Scand 1978;57:403.
2. Karim SMM, Trussell RR, Hillier K, et al. Induction of labor with prostaglandin F_2. J Obstet Gynaecol Br Commonw 1969;76:169.
3. Keirse MJNC, Turnbull A. E Prostaglandins in amniotic fluid during late pregnancy and labour. Br J Obstet Gynaecol 1973;80:970.
4. Keirse MJNC, Flint A, Turnbull A. F prostaglandins in amniotic fluid during pregnancy and labour. Br J Obstet Gynaecol 1974;81:131.
5. Keirse MJNC, Turnbull AC. The fetal membranes as a possible source of amniotic fluid prostaglandins. Br J Obstet Gynaecol 1976;83:146.
6. Casey ML, Cutrer SI, Mitchell MD. Origin of prostanoids in human amniotic fluid: the fetal kidney as a source of amniotic fluid prostanoids. Am J Obstet Gynecol 1983;147:547.
7. Mitchell MD, Bibby J, Hicks BR, et al. Specific production of prostaglandin E_2 by tissues from human uterus and fetoplacental unit. Prostaglandins 1978;15:377.

8. Skinner K, Challis JRG. Changes in the synthesis and metabolism of prostaglandins by human fetal membranes and decidua at labor. Am J Obstet Gynecol 1985; 151:519.
9. Neider J, Augustin W. Increase of prostaglandin E and F equivalents in amniotic fluid during late pregnancy and rapid PGF elevation after cervical dilatation. Prostaglandins Leukotrienes Med 1983;12:289.
10. McCoshen JA, Tagger O, Wodzicki A, et al. Choriodecidual adhesion promotes decidual prolactin transport by human fetal membrane. Am J Physiol 1982;243:R552.
11. Schwartz AL, Forster CS, Smith P, et al. Human amniotic metabolism. I. In vitro maintenance. Am J Obstet Gynecol 1977;127:470.
12. Dubin NH, Ghodgaonkar RB, King TM. Role of prostaglandin production in spontaneous and oxytocin-induced uterine contractile activity in in vitro pregnant rat uteri. Endocrinology 1979;105:47.
13. Okayaki T, Casey ML, Okita JR, et al. Initiation of human parturition. XII. Biosynthesis and metabolism of prostaglandins in human fetal membranes and uterine decidua. Am J Obstet Gynecol 1981;139:373.
14. Schwarz BE, Schultz F, MacDonald PC, et al. Initiation of human parturition. III. Fetal membrane content of prostaglandin E2 and F2 precursor. Obstet Gynecol 1975;46:564.
15. Mitchell MD, Flint APF, Bibby J, et al. Rapid increases in plasma prostaglandin concentrations after vaginal examination and amniotomy. Br Med J 1977;2:1183.
16. Manabe Y, Okazaki T, Takahashi A. Prostaglandins E and F in amniotic fluid during stretch-induced cervical softening and labor at term. Gynecol Obstet Invest 1983;15:343.
17. Guzick DS, Winn K. The association of chorioamnionitis with preterm delivery. Obstet Gynecol 1985;65:11.

A prospective study to compare serum human placental lactogen and menstrual dates for determining gestational age

Paul G. Whittaker, D.Phil., Tom Lind, M.B., B.S., and Jacqueline Y. Lawson, B.Sc.
Newcastle upon Tyne, England

In a group of 575 healthy pregnant women with certain menstrual dates the estimation of the length of gestation from maternal serum human placental lactogen concentrations has been compared with gestational age calculated from the last menstrual period and ultrasonic measurements of the fetal biparietal diameter. In 412 of these patients labor started spontaneously, and the estimated dates of delivery determined by these three methods were also compared. In the range of 9 to 17 weeks of pregnancy, gestational age can be determined by human placental lactogen measurement to within 7 days (±1 SD) which compares favorably with other methods. Regarding the prediction of the expected date of delivery, 88% were delivered within 2 weeks of the date predicted by last menstrual period, 82% within 2 weeks of the sonar date, and 80% by the date determined by human placental lactogen assessment. Prediction of delivery in a further group of 139 women with uncertain dates gave 73% within 2 weeks by sonar date and 69% within 2 weeks by human placental lactogen determination. We suggest human placental lactogen measurements should become part of routine antenatal care complementing rather than replacing the role of ultrasonic scanning. For those doctors and patients who wish to avoid more exposure to ultrasonic scanning than absolutely necessary, human placental lactogen estimates offer an alternative method for assessing the length of gestation. (Am J Obstet Gynecol 1987;156:178-82.)

Key words: Gestational age, human placental lactogen, ultrasound

One of the major facts the obstetrician needs for the management of a pregnancy is an accurate knowledge of the length of gestation. Traditionally this is calculated from the first day of the last menstrual period, and for women in whom this date is known with certainty and whose cycles are regular, such calculations are reasonably precise.[1, 2] Unfortunately some women are unable to remember accurately the date of their last menstrual period, others have irregular menstrual cycles, and for some patients the date offered represents withdrawal bleeding after discontinuation of the use of oral contraceptive agents, making its relation to ovulation less predictable; in such circumstances alternative methods for determining the duration of pregnancy must be used. Ultrasonic measurements of various fetal parameters such as the crown-rump length,

From the MRC Human Reproduction Group, University Department of Obstetrics and Gynaecology, Princess Mary Maternity Hospital, and Department of Clinical Biochemistry, Royal Victoria Infirmary.
Received for publication June 1, 1986; accepted July 13, 1986.
Reprint requests: Paul G. Whittaker, University Department of Obstetrics and Gynaecology, Princess Mary Maternity Hospital, Newcastle upon Tyne NE2 3BD, England.

Table I. Regression analysis of maternal serum human placental lactogen (hPL) levels measured at the first antenatal visit

	Index group	*All patients with reliable dates*
No. of patients	139	585
Equation	Day = 62.7 + 69.4 (hPL)	Day = 63.8 + 65.0 (hPL)
Correlation	0.854	0.776
SD of estimate (days)	7.3	8.4
Mean gestation (days)	86 ± 14	84 ± 13
% within ±7 days of mean	38	44
Range of gestations from last menstrual date (days)	44-132	44-132
Mean hPL (mU/L)	0.335 ± 0.172	0.308 ± 0.159
Lower limit of hPL prediction (days)	0.007-63	—
Upper limit of hPL prediction (days)	0.85-122	—

the biparietal diameter, or the length of the femur have proved to be useful but their relation to gestational age is dependent on the stage of pregnancy at which such measurements are made[3] and the skill and experience of the operator. In addition the equipment is expensive to buy and maintain, and patients may have to travel long distances to reach such facilities.

The pregnancy-specific hormone human placental lactogen is detectable in maternal blood soon after implantation and increases some 300-fold in concentration during the first trimester.[4] In a preliminary study we reported that concentrations of human placental lactogen between 0.01 and 0.8 μU/ml could be used to determine gestational age to within 6.3 days (SD); these concentrations approximate for the first trimester.[5] The assay has since been introduced as a routine clinical service for all patients booking at this hospital; since it is the practice of offer ultrasonic scanning to every patient at about 16 to 18 weeks of pregnancy, we have been able to compare estimates of gestational age from human placental lactogen measurements both with those calculated from the last menstrual period and by ultrasonic measurements of the biparietal diameter.

Patients and methods

Assays of human placental lactogen were undertaken on 886 consecutively booked patients, of whom 49 (5.5%) spontaneously aborted, 19 (2.1%) were delivered elsewhere, and 31 (3.5%) had human placental lactogen concentrations at booking outside of our working range. Nine patients had twin pregnancies, and their data have been analyzed separately, leaving 778 patients who, while displaying the usual spectrum of obstetric outcomes, either had a live birth or were delivered after 28 completed weeks. In this group 585 (75.2%) mothers had reliable menstrual data and 193 did not.

Radioimmunoassay. The assay was based on materials supplied by RIA (UK) Ltd. (Washington, Tyne and Wear, England) as its "hPL early gestational age" kit. The standards were prepared from the World Health Organization standard 73/545 (1 mU = 1 mg) and ranged from 0.007 to 0.85 mU/L; normal male serum was added to standards to counteract nonspecific serum effects. Standards and undiluted samples were incubated with antiserum and iodinated human placental lactogen for 30 minutes at room temperature. A mixture of polyethylene glycol and preprecipitated second antibody was then added, and after a further 15 minutes the samples were processed and counted. Within-assay variation was 2.1% at 0.132 mU/L (n = 10) and across-assay variation was 7.6% at 0.167 mU/L (n = 22).

Sonar scanning. The machine used was a GL Diasonograph NE4200 modified for use with a real-time 3.5mHz head and a 4-bit and 6-bit digital scan converter.

Statistics. Statistical tests for comparison of groups and analysis of linear regression were as described by Armitage.[6]

Results

Reliable dates: human placental lactogen findings

Index group: Initially very strict patient criteria were defined for the creation of an index group in which the relation of serum human placental lactogen to gestational age (in days) could be determined. Each woman had to be able to provide a written record of her last menstrual period, to have a cycle length not exceeding 44 days,[7] to have a sonar assessment of gestational age from the fetal biparietal diameter within 12 days (that is, ±2 SD) of that calculated from the last menstrual period, and finally to have a live, healthy baby following the spontaneous onset of labor at term (defined as 37 to 41 completed weeks).[8] With use of the present kit the range for serum human placental lactogen was optimized at 0.007 to 0.85 mU/L so that the woman also had to have values within this range. There were 139 patients who fulfilled all of these criteria, and linear regression analysis of their data showed that gestational length could be calculated to within 7 days (1 SD) of the last menstrual period date; the earliest estimate of gestation possible was 63 days, with an upper limit of

Table II. Estimation of gestation length from maternal serum human placental lactogen concentrations (585 patients) and ultrasonic measurements of the fetal biparietal diameter (575 patients), relative to that determined from the last menstrual date*

Distribution (days)	*Human placental lactogen (%)*		*Sonar (%)*	
≤6	63	91	63	90
7-13	28		27	
14-20	6		9	
21-27	3		1	
≥28	0.3		0.4	

*Differences in human placental lactogen dates versus differences in sonar dates: $r = 0.5$ ($p < 0.001$). Mean difference (calculated from last menstrual dates): human placental lactogen, 0.3 ± 8.4 (NS); sonar, -4.6 ± 6.6 ($p < 0.001$). The negative bias in the sonar dating occurred because the regression line used applied to the whole range of pregnancy and was not optimized to gestation limited to 17 to 19 weeks.

122 days. The details of the analyses are shown in Table I.

Reliable dates group: The index group was now widened to include those who had reliable menstrual dates, a cycle length not exceeding 44 days, a serum human placental lactogen concentration inside the working range, and in whom the outcome was anything other than spontaneous abortion or twins. The sonar findings were not taken into account. This increased the group to 585 (19 women had been excluded because their human placental lactogen values were outside the working range). The mean gestation at booking for the group as a whole was 12 weeks (±2 weeks; 1 SD), ranging from 6 to 19 weeks. The linear regression calculated for this group did not differ significantly from that of the index group (Table I). Use of a log transformation of human placental lactogen values made the fit somewhat worse ($r = 0.744$) and using a quadratic function gave only a marginal improvement ($r = 0.781$). It was thus decided to use the regression line determined from the index group for all further comparisons.

One way of comparing how well human placental lactogen assessment performs in a group of individuals is to subtract the length of gestation calculated by means of the last menstrual period from the length of gestation determined by human placental lactogen. There will be some underestimates and some overestimates, but with a large group the mean of all of the differences should not be significantly different from zero and should have a small standard deviation. This proved to be the case for the 585 women with reliable menstrual dates; the mean diference was 0.3 days, with a standard deviation of 8.4 days. The distribution of gestational estimates by human placental lactogen was such that 91% were within 13 days of their last menstrual period date (Table II). Calculations of gestational age from measurements of human placental lactogen at the extremes of the assay range did not reveal any systematic bias.

Table III. Estimation of expected date of delivery in 412 spontaneous deliveries with *reliable* menstrual dates

Distribution (days)	*Last menstrual date (%)*		*Sonar (%)*		*Human placental lactogen (%)*	
≤6	58	88	53	82	49	80
7-13	30		29		31	
14-20	6		10		12	
21-27	1		3		4	
≥28	5		5		4	

Table IV. Estimation of expected date of delivery in 139 spontaneous deliveries with *uncertain* menstrual dates

Distribution (days)	*Sonar (%)*		*Human placental lactogen (%)*	
≤6	47	73	40	69
7-13	26		29	
14-20	15		19	
21-27	5		6	
≥28	7		6	

Reliable dates: ultrasonic findings

Of the 585 women in whom human placental lactogen data were available, 575 also had ultrasonic measurements of the biparietal diameter. Unlike the human placental lactogen determinations the ultrasonic measurements could not be undertaken blind because the operators knew the patients had deliberately been asked to come at 16 to 18 weeks' gestation; indeed the mean gestation from last menstrual period at scanning for the whole group was 17.5 weeks, and 72% were seen within 2 weeks of the mean. Use of the difference between the ultrasonically determined length of gestation and that derived from the last menstrual period showed that 90% of the mothers were within 2 weeks of the gestation calculated from the menstrual date (Table II).

For the estimation of date of delivery the measurements of 412 patients who had certain last menstrual period dates as well as both human placental lactogen and ultrasonic data and who went into spontaneous labor (mean, 278 days' gestation) were analyzed. Calculated from the last menstrual period the percent of patients delivered within 2 weeks of the expected date of delivery was 88%, from sonar determinations it was 82%, and from human placental lactogen determinations it was 80%. The distributions are given in Table III.

Uncertain dates. A further patient group was com-

posed of 220 women for whom there was reason to doubt the accuracy of their menstrual dates. In 124 cases the mothers could not remember their dates, a further 83 said the date they gave represented withdrawal bleeding from oral contraception, and 13 women had such irregular cycles their date was an unreliable index to ovulation. In this group 146 women experienced spontaneous onset of labor after uncomplicated pregnancies and delivered live healthy babies; of these, 139 (95%) had human placental lactogen values within our working range. We compared their expected date of delivery determined both from human placental lactogen concentrations and from sonar results with the actual date of delivery; 69% were delivered within 2 weeks of the dates predicted by human placental lactogen, and 73% were delivered within 2 weeks of the date predicted by ultrasonic scanning (Table IV).

Twins. There were nine pairs of twins in the series and the human placental lactogen/last menstrual period gestation differences in this group averaged 8 days (±14 days; 1 SD). The numbers are too few to draw conclusions, but the apparent trend to overestimate gestation is probably a function of the small number in this group.

Spontaneous abortions. There were 39 women who spontaneously aborted and the mean gestational difference in this group was −5.3 (±14.3; 1 SD) days, which just achieved significance ($p < 0.05$). This could have been anticipated from the endocrine changes known to occur before spontaneous abortion.

Comment

It has been suggested that the maternal serum concentrations of human placental lactogen, human chorionic gonadotropin and Schwangerschaft's protein can be used to determine the length of pregnancy during the first and second trimesters of pregnancy. The use of human chorionic gonadotropin appears to be reliable,[9] but its clinical usefulness is limited to <10 weeks because after this time maternal serum levels decline. Both human placental lactogen and Schwangerschaft's protein increase steadily throughout pregnancy[10] and appear to give clinically useful estimates of the length of gestation during the first and second trimesters,[5, 11, 12] but these studies involved relatively small numbers of patients. In this investigation a large number of patients have been studied, embracing the usual range of obstetric outcomes and the human placental lactogen estimates of the length of pregnancy compared not only to last menstrual period data but to sonar estimates of gestation and the actual date of delivery in those women undergoing spontaneous labor.

Our original series had been composed of women attending our research unit who had usually attended the antenatal clinic before 10 weeks' gestation.[5] The present large population attended for the first time over a much wider range of pregnancy, but 97% of those with certain menstrual dates had human placental lactogen values within our original range.[5] A new regression line was calculated from the present index group because of the change from an in-house human placental lactogen assay to a commercial kit; the standard deviation was 7 days and, when applied to everyone with reliable dates, placed 91% within 2 weeks of their last menstrual period date. Our routine clinical ultrasonic service for the same group yielded a standard deviation of 6 days and placed 90% within 2 weeks of the last menstrual period dates. It must be borne in mind that the sonar measurements were made with full knowledge that the patients were approximately 16 to 18 weeks pregnant whereas the human placental lactogen determinations were blind and made over a much wider range of weeks.

It has been suggested that one way of checking the accuracy of gestational assessment would be to compare the actual date of confinement against that predicted by any particular assessment technique. Campbell et al.[13] reported that 89% of patients delivered within 2 weeks of the ultrasonically predicted date of delivery, whereas in the same group only 85% delivered within 2 weeks of the last menstrual period predicted date. Ahmed and Klopper[12] used a similar argument. In our series of the 412 women with reliable dates who went into spontaneous labor, 88% were delivered within 2 weeks of the date predicted by last menstrual period (that is, the date of onset of the last menstrual period plus 280 days), 82% within 2 weeks of the ultrasonic date, and 80% by the date derived by human placental lactogen. While it is of interest that data from different sources are so similar, we feel this form of analysis to have little clinical relevance; many factors influence the time at which an individual woman will go into spontaneous labor, and 280 days is not a rigid interval. The work of Guerrero and Florez[1] showed that the interval from ovulation to spontaneous delivery had a standard deviation of 12 days.

A group of patients of particular clinical concern are those with uncertain dates: 25% in our study. Of these, 95% had serum human placental lactogen levels within our range when they first attended the antenatal clinic, and all had a sonar estimation of biparietal diameter. Estimation of date of delivery in these women was similar for both methods, some 70% delivering within 2 weeks of the predicted expected date of delivery.

In our experience dating the length of gestation by human placental lactogen levels and the sonar estimate of the biparietal diameter are equally reliable. It could be argued that use of sonar determinations of the fetal

crown-rump length would allow a more precise assessment of gestational length, but this was not the experience in our previous though smaller series.[5] The standard deviation of the crown-rump length/last menstrual period gestational determinations was ±6.1 days which was not significantly different from the ±6.6 days reported here for biparietal diameter estimates. This is not to suggest that the estimation of human placental lactogen should replace sonar determinations but that both methods have a role to fulfill. Pregnant women have blood sampled at their first antenatal visit for a variety of tests so the cost of taking the blood sample for human placental lactogen in terms of syringes, needles, and the manpower required is shared across this range of tests. Since the hormone is stable in serum at room temperature, the sample could be sent to a central laboratory from a wide area and a technician could process hundreds of samples per week. Finally, immunoassay equipment is available in many laboratories and their capital cost is spread over the many hormone assays requested by doctors in different disciplines. Ultrasonic machines used for obstetric scanning are often dedicated to that purpose, are expensive to buy and maintain, and require highly experienced staff to use them and interpret the findings; moreover, the patients usually have to travel to the facility. For those hospitals performing a sonar scan at booking for gestational assessment and repeating the scan at about 18 weeks for fetal anomaly detection, the estimate of human placental lactogen could offer a reliable and cheaper alternative to the first scan. It would also offer flexible clinical management: Every patient could be offered an human placental lactogen assessment at booking, and for those in whom there was a discrepancy between the last menstrual period and human placental lactogen estimates, a sonar scan could be arranged.

Why use assay methods at all if the sonar method is reliable? Some reasons have been discussed above, but a major reason is the 20% or so of women who have no menstrual date to offer. In such circumstances rather than the sonar estimate being used alone for dating, a second diagnostic system is now available. There are also circumstances in which women with apparently reliable menstrual dates have a sonar-derived gestational age at variance with these dates; an independent human placental lactogen assessment would obviously be useful in such circumstances. Finally, for those places that do not have ready access to a sonar service, human placental lactogen assay could offer a cheap centralized facility for assessing gestational age. For these reasons we suggest human placental lactogen measurements should become part of routine antenatal care, complementing rather than replacing the role of ultrasonic scanning.

We are grateful to RIA (UK) Ltd., Washington, England, for its contribution toward this project. The assistance of Jean Gerrard, Thelma Scholefield, and Mary Maxwell with data collection and analysis is also appreciated.

REFERENCES

1. Guerrero R, Florez PE. The duration of pregnancy. Lancet 1969;2:268.
2. Royston JP. Basal body temperature, ovulation and the risk of conception with special reference to the lifetimes of sperm and egg. Biometrics 1982;38:397.
3. Yagel S, Adoni A, Oman S, et al. A statistical examination of the accuracy of combining femoral length and biparietal diameter as an index of fetal gestational age. Br J Obstet Gynaecol 1986;93:109.
4. Aspillaga MO, Whittaker PG, Taylor A, et al. Some new aspects of the endocrinological response to pregnancy. Br J Obstet Gynaecol 1983;90:596.
5. Whittaker PG, Aspillaga MO, Lind T. Accurate assessment of early gestational age in normal and diabetic women by serum human placental lactogen concentration. Lancet 1983;1:304.
6. Armitage P. Statistical methods in medical research. Oxford, England: Blackwell Scientific Publications, 1971.
7. Vollman RF. The menstrual cycle: major problems in obstetrics and gynecology. Eastbourne, England: WB Saunders, 1977:19.
8. Working party to discuss nomenclature based on gestational age and birth weight. Arch Dis Child 1970;45:730.
9. Lagrew DC, Wilson EA, Fried AM. Accuracy of serum hCG concentrations and ultrasonic fetal measurements in determining gestational age. Am J Obstet Gynecol 1984;149:165.
10. Braunstein GD, Rasor JL, Engvall E, et al. Interrelationships of hCG, hPL, and PSBG throughout normal human gestation. Am J Obstet Gynecol 1980;138:1205.
11. Peeters LL, Lemons JA, Niswender GD, et al. Serum levels of hPL and hCG in early pregnancy: a maturational index of the placenta. Am J Obstet Gynecol 1976;126:707.
12. Ahmed AG, Klopper A. Estimation of gestational age by last menstrual period by ultrasound scan and by SP1 concentration: comparisons with date of delivery. Br J Obstet Gynaecol 1986;93:122.
13. Campbell S, Warsop SL, Little D, et al. Routine ultrasound screening for the prediction of gestational age. Obstet Gynecol 1985;65:613.

Similarities of Negro and Caucasian fetal extremity lengths in the interval from 9 to 20 weeks of pregnancy

Robert A. Munsick, M.D., Ph.D.
Indianapolis, Indiana

Foot, leg, and arm measurements were compared from 599 electively aborted fetuses of Negro and Caucasian women who had reliable menstrual dates and agreement between dates and uterine size. When compared by week, no significant racial differences were detected in the means of any of these measurements between 9 and 20 menstrual weeks of pregnancy. The combined, corrected data provided smoother curves than those previously published. The following formulas are recommended for weeks 9 to 20 of pregnancy, where W = weeks and F, L, and A are the foot, leg, and arm measurements in centimeters: $W = 4.5261 + 11.937(F) - 4.4895(F^2) + 0.71583(F^3)$; $W = 5.9957 + 5.7279(L) - 1.2246(L^2) + 0.11880(L^3)$; $W = 5.4007 + 5.0305(A) - 0.93540(A^2) + 0.079022(A^3)$. (Am J Obstet Gynecol 1987;156:183-5.)

Key words: Fetus, extremity, pregnancy, race, foot, leg, arm

Recently I[1] showed that the fetal foot length data of Streeter, published in 1920, and of Hern, published in 1984, may be properly used as a "gold standard" of fetal age. However, the proportions of Negro and Caucasian mothers that underwent abortion were not known. Assuming that there were no significant racial differences, the mixed racial data were combined. (See reference 1 for additional sources.)

Jakobovits et al.[2] studied the foot and crown-rump lengths of black, white, and Central American fetuses, but because of poor menstrual dates they found it necessary to present foot lengths in terms of crown-rump lengths. They concluded that if there were racial differences in foot lengths they were probably negligible.

The purpose of this study was to determine whether there are racial differences in the foot, leg, or arm lengths of the fetuses of Negro and Caucasian mothers in the interval from 9 to 20 menstrual weeks of gestation and, if differences were not demonstrated, to improve with additional data the previously published regression equations.[1]

Material and methods

In my previous report,[1] race was not included in the data output to the computer sequential files. Therefore, the original records were compared with the mothers' charts, and when race was identified it was recorded. New cases with racial data were added to the original ones. Criteria for inclusion in the study were as follows: The mother was identified as black or white, her menstrual dates were accurate, at least one of the three fetal measurements was recorded, and the vaginal estimate of uterine size agreed within 2 weeks of dates. Measurements of the foot (heel to extended longest toe), leg (top of the flexed knee to the bottom of the calcaneus), and arm (top of the flexed elbow to the longest extended finger) were made with vernier calipers immediately after dilatation and suction or evacuation.

For simple statistical computations, such as mean, SD, and Student's *t* tests, programs were written in N-Basic for the NEC 8001-A personal computer with the use of standard formulas as previously described.[1] Polynomial regression equations for weeks, based on foot, leg, and arm lengths, were solved with the SAS statistical package of SAS Institute Inc., Box 8000, Cary, NC 27511-8000. Because most variability of dated fetal extremity measurements is thought to be caused by inconsistent times of fertilization within the menstrual cycle and poor recall of menstrual dates, ideal curves were sought by eliminating all measurements at each week that varied from the mean by more than 2 SD.

No information was obtained concerning the incidence of fetuses of mixed races. Although such pregnancies were undoubtedly included in this series, their incidence was likely well below 10%, as it is in our general obstetric populations of black and white mothers. Hence the presence of some mixed-race fetuses should not have materially influenced the results. I will therefore refer to fetal race on the basis of maternal race. Similarly, no attempt was made to define race narrowly; it was judged by the appearance of the mother. Other races were excluded.

From the Department of Obstetrics and Gynecology, Indiana University School of Medicine.
Received for publication January 3, 1986; revised June 26, 1986; accepted July 10, 1986.
Reprint requests: Robert A. Munsick, M.D., Ph.D., Department of Obstetrics and Gynecology, Indiana University School of Medicine, 926 West Michigan St., Indianapolis, IN 46223.

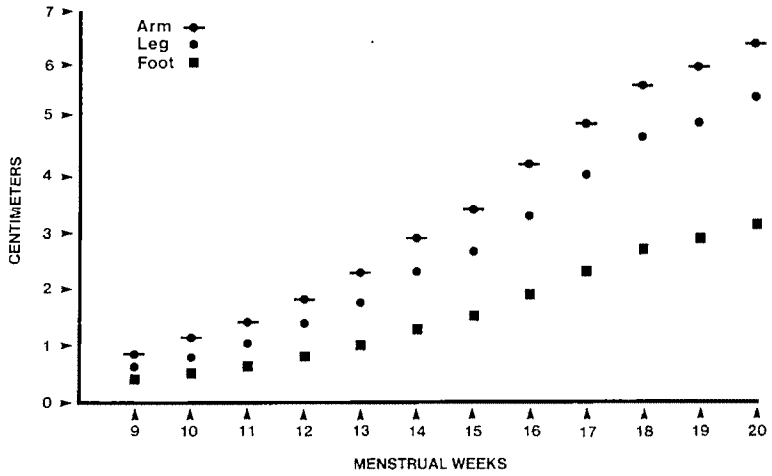


Fig. 1. Recommended, corrected fetal extremity lengths.

Table I. Combined, corrected data: Recommended values from previous and current studies

Weeks' gestation	*Foot*				*Leg*			*Arm*	
	S	*H*	*M*	*Current*	*H*	*M*	*Current*	*M*	*Current*
9	0.46	—	0.46	0.46	—	0.63	0.63	0.84	0.84
10	0.55	0.6	0.56	0.56	0.8	0.80	0.80	1.11	1.11
11	0.69	0.7	0.69	0.69	1.1	1.09	1.09	1.47	1.47
12	0.91	0.8	0.89	0.87	1.3	1.45	1.41	1.93	1.85
13	1.14	1.0	1.13	1.07	1.7	1.93	1.80	2.50	2.33
14	1.40	1.4	1.39	1.33	2.4	2.43	2.35	3.11	2.93
15	1.68	1.8	1.68	1.55	3.1	2.88	2.70	3.71	3.45
16	1.99	2.1	2.12	1.94	3.6	3.67	3.36	4.52	4.24
17	2.30	2.3	2.41	2.31	4.0	4.16	4.02	5.14	4.97
18	2.68	2.5	2.72	2.70	4.3	4.68	4.71	5.68	5.64
19	3.07	3.0	2.98	2.90	5.1	5.24	4.99	6.23	5.97
20	3.33	3.3	3.17	3.17	5.6	5.43	5.43	6.39	6.39

S = Streeter, H = Hern, M = Munsick.[1] All measurements in centimeters.

Of the original 575 patients reported on,[1] racial data were obtained for 522. Seventy-seven new cases were introduced, bringing the total to 599.

Results

Mean extremity lengths and SDs were determined for black and white fetuses at 9 to 20 menstrual weeks. Means and variability were very similar to the values previously published.[1] With the use of Student's *t* test, no significant differences were demonstrated between any of the mean extremity measurements in white and black fetuses for any of the weeks studied (all p values >0.1; complete data are available on request).

Because significant racial differences were not demonstrated, the data were combined by week and corrected by removing all cases that departed from the combined mean by ≥2 SD. These idealized means are shown in Table I along with some of the data from Table II of my first article.[1]

With the use of these data, polynomial regression formulas were derived for foot, leg, and arm lengths. Tests of the significance of the intercepts and first through third- or fourth-order regression coefficients showed a p value of <0.002 for all third-order polynomial equations but lack of significance for all fourth-order equations. Hence third-order equations were adopted. Adjusted R^2 values for these were 0.9989 for foot, 0.9981 for leg, and 0.9992 for arm lengths. Curves of idealized fetal extremity growth with weeks of gestation are shown in Fig. 1. These are smoother than those shown in the last article.[1]

The polynomial equations to determine menstrual weeks (W) from foot (F), leg (L), and arm (A) measurements in centimeters are:

$$W = 4.5261 + 11.937(F) - 4.4895(F^2) + 0.71583(F^3)$$

$$W = 5.9957 + 5.7279(L) - 1.2246(L^2) + 0.11880(L^3)$$

$$W = 5.4007 + 5.0305(A) - 0.93540(A^2) + 0.079022(A^3)$$

Maximum errors in weeks, according to these equa-

tions and the corrected data in Table I from which they were derived, follow: for foot, −0.2 weeks at 19 weeks; for leg, +0.24 weeks at 14 weeks; and for arm, +0.2 weeks at 18 weeks. With the use of differential calculus, it was found that the greatest rates of growth, expressed in centimeters per week, were 0.39 at 16.4 weeks for foot, 0.66 at 16.1 weeks for leg, and 0.75 at 15.5 weeks for arm length.

The following naperian logarithmic formulas are acceptable but are less accurate than the polynomial formulas shown above at weeks 9, 19, and 20, especially for the leg and arm:

$$\ln(W) = [\ln(F) + 2.92247/0.434294]/2.65803$$

$$\ln(W) = [\ln(L) + 3.03217/0.434294]/2.95033$$

$$\ln(W) = [\ln(A) + 2.70467/0.434294]/2.75733$$

With the use of these logarithmic formulas, maximum errors in weeks were: for foot, +0.39 at 9 weeks and −0.59 at 20 weeks; for leg, −0.62 at 19 weeks and −1.09 at 20 weeks; and for arm, −0.71 at 19 weeks and −1.25 at 20 weeks.

Comment

The finding that race (Negro and Caucasian) does not significantly affect the fetal foot, leg, or arm length between 9 and 20 weeks adds confidence to the mixed racial data previously reported.[1] These conclusions should not be extended beyond 20 weeks or to races other than Caucasian and Negro.

For programmable calculators, the third-order polynomial equations yield quicker and apparently more accurate results than the fourth-order formulas previously presented.[1] We have found that it is frequently possible to obtain ultrasonic measurements of fetal extremities in the second and late first trimesters and that applying the above formulas to these parameters agrees very well with age estimates derived from biparietal, crown-rump, and femur measurements.

I am again indebted to Pao-Lo Yu, Ph.D., Department of Medical Genetics, Indiana University School of Medicine, for his statistical advice and review of this manuscript.

REFERENCES

1. Munsick RA. Human fetal extremity lengths in the interval from 9 to 21 menstrual weeks of pregnancy. AM J OBSTET GYNECOL 1984;149:883.
2. Jakobovits A, Westlake W, Iffy L, et al. Early intrauterine development. II. The rate of growth in black and Central American populations between 10 and 20 weeks' gestation. Pediatrics 1976;58:833.

Academia—An endangered species

Douglas J. Marchant, M.D.
Boston, Massachusetts

To understand the present crisis in American medicine, it is necessary to review the relevant medical history of the nineteenth century. Only in very recent times have physicians, as a class, enjoyed a favored economic status. Medicine had been a poorly paid profession. Initially the American Medical Association waged battle against the cultists, many of whom were financially quite successful in contrast to the regular physicians. Increasing numbers of poorly educated physicians entered practice, and education reform became a major goal of the American Medical Association.

From the Departments of Obstetrics and Gynecology and Surgery, Tufts University School of Medicine.
Presidential address, presented at the Fourth Annual Meeting of the Gynecologic Urology Society, Palm Beach, Florida, October 2, 1984.
Received for publication January 16, 1986; revised July 8, 1986; accepted July 22, 1986.
Reprint requests: Douglas J. Marchant, M.D., 171 Harrison Ave., Boston, MA 02111.

The complaint that there were too many doctors was associated with the realization that there were too few paying patients. The only patients who entered the hospitals were the indigent sick. Hospitals began as charitable institutions and until the middle of the nineteenth century served only the sick poor who needed bed care. Special institutions called dispensaries, whose origins go back to the eighteenth century, provided ambulatory care for "the deserving sick poor." As hospitals and dispensaries developed under a tradition of charitable care, the community and philanthropists provided the funds and saw to it that the institutions were run economically and efficiently. Physicians provided medical care without charge. Theoretically this was the ideal system. However, even a hundred years ago, things did not work out well. What level of poverty described the needy? For the totally destitute, there was no problem. Could the sick or poor afford to pay for medical care, and if they could, why should they not do so?

The beginnings of an adversary relationship between

the physician, the hospital, and the university began in the late nineteenth century in a nation ill equipped to deal with the social problems of the time. Dispensaries and later outpatient hospital departments accepted all comers, and many practitioners complained about the loss of patients to the dispensary.

With the decline of the dispensary, hospital outpatient departments provided comparable functions. In 1916 it was estimated that there were a total of 900 dispensaries, of which 645 were outpatient departments of hospitals.[1] According to Vietor,[2] in 1886 it was estimated that about one fourth of the population of Boston and about half the population of New York were receiving free medical treatment. In 1905 Gay[3] declared that "as a matter of public record three hundred thousand patients in Boston received practically free treatment—about half the population of the city." Obviously the practicing physician had a legitimate complaint if people who could afford private care were taking advantage of the available free care. On the other hand, some physicians viewed the dispensary as an excellent way of acquiring experience and improving their skills. The dispensaries often served as a teaching function for medical schools. Thus a free dispensary attached to a medical school might drain patients away from the private practice of the community. Dispensaries provided clinical material for the training of specialists and as a result attracted the ambitious young men who wanted training and were glad to serve in the dispensaries to get it. The dispensaries in addition maintained laboratories, and the physicians relied on "instruments of precision"—what we now call "tests." Family physicians were relatively slow in assimilating this new knowledge, and even at this early stage the differences between the services of a clinic and a private physician were obvious.

Medical advances in the latter part of the nineteenth century produced a different kind of abuse—hospital abuse, quite different from the dispensary setting. This problem had to do with patient bed care rather than ambulatory care. As I mentioned earlier, bed care in hospitals for many years was a charitable offering, and middle-class families tried to avoid this. Obviously, in addition to the stigma of a hospital admission, there was the higher death risk because of infection and other iatrogenic problems. However, because of developments in medicine, particularly the science of bacteriology and the growth of laboratory medicine, hospitals began to attract patients who could pay and therefore started to charge such patients for bed, board, and nursing services. The attending physicians, however, did not share in the subsidy. Well-to-do patients could enter the hospital, pay for their room and board and nursing, and receive excellent medical care, all without paying the attending physician. The physicians, not without reason, considered this "hospital abuse." To correct the situation, some hospitals raised no objections to the covert payment of private fees. Some physicians, especially surgeons, established their own hospitals. Many large charity hospitals established a private pavilion where staff members could take their private patients.

This process at the turn of the century marked a major transformation in medical practice. Certain aspects became more and more hospital based rather than home based. Obviously those physicians who admitted private paying patients to their service encouraged a system in which the hospital was being made directly to subserve the private interests of the individual physicians. The staff who received fees had an unfair advantage over those members of the medical community without hospital appointments—the beginnings of the "town versus gown" controversy. Doctors associated with medical schools and hospitals had privileges of hospital admission for their patients to achieve more precise diagnosis and more successful treatment. The referring physician or the family doctor was undergoing a diminution in stature. The complexities of medical education encouraged by the American Medical Association, social stratification of the community, and economic developments, all contributed to the formation of academic medicine or a medical elite.

Enter the "lodge practice." Because there continued to be too many doctors and because the younger physicians had to make a living somehow, many joined a form of prepaid health care, in which organized groups hired a physician to provide medical service to the members of that group and their families. In the east side of New York alone in 1910, it is estimated that there were 1500 to 2000 lodges. If one considers that the average income of a physician in 1910 was $2000, the young physician employed by a lodge with several hundred members would have a very good start in practice indeed.

Knapp[4] described this practice as "lowering the profession to the level of a trade," and certainly we have heard this in our day. Clearly not all physicians were opposed to this lodge practice. Some would gain a wide clinical experience much as their predecessors in the dispensary. Organized medicine, however, had long opposed this type of practice, and in 1911 the American Medical Association published an opinion on various aspects of this subject.[5] However, one physician declared very perceptively that "the unethics of contract practice depends upon the minuteness of the fee." The major questions of the times, and I might add of our time as well, were how could the poor receive adequate medical care and how could the physicians make an adequate living consonant with their educational investment and their specialized skills. As we all know,

the problems expanded, the question of voluntary or compulsory health insurance has become intrusive, and contract health care has taken on a new dimension.

This historical overview provides the perspective to examine contemporary medicine. Let me begin by summarizing the unfortunate case of Dr. Z as reported in *The New England Journal of Medicine* in August 1984.[6] The unfortunate Dr. Z had been stripped of his hospital privileges and was placed on probationary office practice because of "failure to adjust to the new medicine. He refused to accept the guiding principles of the new medicine—that the encounter with the patient, the illness episode—is accurately and completely defined by a disease diagnosis and that for every such disease there is a corresponding technological treatment." Dr. Z insisted that the patients came to him for many reasons other than the presence of a specific disease. He always seemed to be bogged down in the process of health care delivery—in taking care of the patient, as he puts it, instead of stressing outcome. Dr. Z further asserted that "in addition to providing scientific expertise physicians should express concern about their patients, gain their confidence and co-operation and give them and their families peace of mind." The answer, of course, to Dr. Z is that "we have no code or audit criteria for explanation or reassurance or what he called personal care."

Unfortunately, despite mandatory attendance at indoctrination sessions, Dr. Z seemed incapable of understanding and participating in the new mission of hospitals and thus the loss of his hospital privileges. He was never able to think of medicine as an industry and view a hospital as a business. His opposition to the dropping of the teaching program was particularly offensive.

Obviously Dr. Z is wrong. Hospitals are a business. The bottom line is "whether the doctor can contribute to the hospital's overall marketing plan." Cost accounting must be part of physicians' grand rounds. We can no longer look at patients as persons but must view them as output or input or as products. We must learn to manage product lines. Obviously Dr. Z had felt the impact of "monetary disincentives" when he lost his hospital privileges. He was then subjected to thinking adjustment sessions and given training in computer coding, advertising, market analysis, and selling strategy. Unfortunately, he reverted to his "old habits." He clings to the archaic belief that medicine is an ethical rather than a commercial enterprise. Our proposed time frame for developing his new mind set is short. If he cannot adapt to the new medicine, this is too bad, there are plenty coming along who will.

Is medicine still an art? I agree with Truman G. Schnabel who stated in an essay in *The New England Journal of Medicine* "medicine's art lies in a skillful manipulation of the relationship between doctor and patient which when combined with the logical use of medicine's science will lead to the best kind of patient care."[7] Unfortunately, the very achievements in medicine that have provided us not only with clues to disease but the means to cure them have diminished the esteem that physicians had enjoyed for many years. Technology has intruded into the practice of medicine, and the manner in which we deal with patients seems to have become less important. This era of technologies and the use of computers will have a significant impact on the doctor-patient relationship.

Another aspect of medical care that is known to all of you is that patients themselves assume more and more responsibility in the decision-making process. It seems to me that for the correct decision to be made the doctor-patient relationship must be *closer* than ever. The doctor and the patient must be able to communicate in a logical and understandable way and over a considerable period of time. Clearly, with the challenge of patient responsibility the physician must be even more sympathetic and kind and the art of medicine must be as important as its science. That this is not the case is indicated by a 1983 poll commissioned by the American Medical Association that revealed that 62% of persons in this country are losing faith in physicians.[8] Responders indicated that physicians are too interested in money and not interested in their patients. For the specialist the process of a correct diagnosis becomes almost an end in itself. Actually, the patient only wants to feel better. The message patients receive is that of an impersonal, technically dominated medical care system. The fact that the health care provided may be improved does not seem to be as important as what might be considered a "hidden message" that the machine has become the physician, the definitive advisor. Predictably this leads to the development of the "too ept" physician. We have peer review to cover the inept physician, but I believe we need a different charge to these committees that will judge the too ept—that is, the medical zealots. For those of us dealing with cancer patients it is clear that we need a new understanding of medical technology. As Crawshaw[9] said so well "by curious inversion of human values, scientific therapeutic zeal has become a new dangerous iatrogenic disease—the technical fix. Caught up in the wonderment of burgeoning technology, physicians become blind to the difference between physiology and life." This technical fix is becoming widespread. Unless we are careful there will be an irreversible distortion of our humanity.

Before discussing the changing monetary aspects of medical care, let me complete the historical review with a few words about the controversy over physician supply. There is some evidence that market forces are responding to a perceived surplus of physicians. Some

medical schools are reducing enrollment, and certainly all medical schools are feeling the decrease in reduced federal funding. On the other side of the ledger is the increasing cost of medical education. A recent American Medical Association survey showed that some 60% of physicians in practice 2 years or less have educational loans outstanding. Additional market forces were recognized by the Rand Corporation that showed that increasing numbers of physicians are locating their practices in smaller communities and that Americans in nearly every community with a population of at least 2500 now have ready access to a physician. Because there is no certain way to predict the future needs of physician manpower, the educational system for physicians should not be locked into a rigid system of quotas or limits. With the market forces now at work the present system will resolve most of the problems associated with the fluctuations in the economy and an increase in physician population. Citizens with freedom of choice rather than centralized planners are more likely to develop and make use of information necessary to allow the market place to function effectively.

The major health manpower issue facing the United States is not an excess of physician supply but an excess of specialist physicians. Because we have so many specialists, we rely on some to do primary care, but because of temperament and training they may not be educationally prepared to function as a generalist physician. In the United States there is an unusually high rate of operations and invasive procedures. There is no waiting list for any procedures, and certainly there is no shortage of surgeons.

Having described the complex relationship between physicians, hospitals, and universities and the social conditions of a hundred years ago that initiated changes in medical practice, it is appropriate to review what can be called the "monetarization" of medical care and entrepreneurism in medicine. A number of observers have expressed alarm at how fast American medicine appears to be turning from a profession into a business. The monetarization of medicine as defined by Ginsberg[10] is the "rapid penetration since 1950 of the money economy into all facets of the health care system." Its influence is reflected in the growth of academic health centers, the shift from voluntary employer physicians in large teaching hospitals, the payment of what appear to be reasonable stipends to house staff, the marked decline in philanthropy in meeting operating deficits, and the substantial reduction in unrequited services by physicians.

In the 1940s, medicine was only partly monetarized. However, rapid expansion in hospital insurance and the enactment of Medicaid programs in 1965 set the stage for the complete monetarization of health care. Although the major impact of this was and remains on the hospital sector, physicians surely are the critical and ultimate providers of care. I believe we cannot view the practice of medicine as just another business undertaking like retailing or banking. To rely on the market to discipline professionals is to overestimate what the market should be asked to do or indeed is capable of doing. There are critics and defenders of this growth of the for-profit sector in medicine. Relman[11, 12] maintains that these profits result from nothing less than the buying and selling of assets and the avoiding of unprofitable activities such as teaching and care for the poor. Defenders predictably emphasize gains from access to more capital, improved planning and operating systems, and more "professional" management.

A further development of this monetarization is the entrepreneurism of medicine exemplified by hospitals organizing a variety of corporations that spin off traditional services and joining or competing with doctors and other hospitals in new ventures such as health maintenance organizations, preferred provider organizations, surgery centers, and clinics in shopping malls. The major difference here is that in the usual business venture investors risk their own money. In this situation, not only is someone else's money being risked, but the very institutions that we depend on to provide services may also be at risk. Part of the problem is the hysteria associated with the high cost of medical care and its perceived shortcomings—however, these services still provide the best medical care available in the world. I believe that the American public will not permit a system that encourages bottom line quality and a price competition environment rather than personal care.

In any system, care of the disadvantaged is the central problem. It is worth remembering that in spite of all the skills that may be developed, including marketing, diversifying, and maneuvering of hospitals, unless these resources accommodate the needs of the unserved and the underserved, the health care system will be overturned no matter how efficiently it may serve those who can afford it. As Cunningham[13] has stated, "there appears to be two possibilities—less competition of the kind of government, industry and some hospitals are pushing—or more federalization. The question is which shall it be?" Many feel that the physician and the health administration will move from the professional model with its traditions to one of professionally regulated competition. Perhaps the eighteenth century philosopher was correct in noting that "competition makes the prince rule more wisely than his wishes alone intend."

An example of the accelerating trend in for profit organization is the transformation of the health maintenance organization movement. The health maintenance organization is one form of alternative health care delivery which features some limitation on a pa-

tient's choice of physician in return for comprehensive benefits provided at a fixed price. It is clear that insurance organizations, hospital management companies, physician entrepreneurs, private business men, and the like are in the process of expanding medical plans that will compete against traditional practitioners. It is interesting that the only organizations that seem to be the least involved in this movement although they are the most threatened are the academic medical centers. One of Wall Street's highly regarded stock analysts has noted that although the health maintenance organization appears to be the ideal solution to cost escalation facing the country, few people have been willing to abandon the idea embedded in the American health care system of unlimited quality and access even when it is no longer cost effective.

There is evidence that the fee-for-service physician is not sitting idly by in the face of this competition. However, it seems certain that life will never be the same again for most practitioners, as a consequence of the new emphasis on greater price sensitivity among third-party payers. Certainly one long-range device for countering this dilemma would be the teaching of medical economics to our medical students, interns, and residents. Few if any medical schools include in their curriculum the crisis of financing medical care and certainly none exposes the doctor as the key culprit.

Let us look now at the relationship between hospital management and its medical staff under a prospective payment system. This, I believe, is the crux to understanding what I feel may be the gradual demise of academic institutions. First, we must understand the traditional relationship between hospital administration and the medical staff. Clearly, hospital management focuses on finances, and its primary goal is to keep the hospital fiscally solvent. The medical staff's primary goal is to provide patients with the best possible medical care with less regard for cost. With the new prospective payment system based on a diagnostic-specific program in contrast to the traditional cost-retrospective reimbursement, hospitals are at greatly increased financial risk. Because physicians control 60% to 70% of the health care expenditures, hospitals must convince the medical staff to adhere to "cost-effective" patterns, and the potential for this relationship between hospital and medical staff to become even more volatile is enormous. Hospital managers must be certain that each physician's pattern of use does not deviate from the narrow range of reimbursement established in each one of the current 467 clinical diagnosis related groups. Some type of sophisticated information system will be required to monitor physician behavior and correlate financial information with clinical information and then assess the cost-effectiveness of the diagnosis related groups singly and comparatively. This is quite reminiscent of the plight of Dr. Z. Once a physician has been identified or appears to exhibit a costly and inefficient behavior "he or she may be approached through education peer pressure or conceivably even restriction of privileges if the costly behavior persists." However sophisticated, aggregate hospital cost accounting does not tell us what a service really costs. Hospitals are looking at *systems* that allow comparison of various diagnoses among multiple hospitals. Cost-effective standards will have to be established for regional and national comparison. Obviously such standards once established will be used to evaluate which diagnosis related groups are cost effective for the particular institution and therefore "profitable to offer." If unprofitable diagnosis related groups are dropped, expensive tertiary services may be the first to go, especially among hospitals in the for-profit sector. Under the new Medicare prospective payment system the educational expenses of hospitals are covered. However, this system poses a distinct danger for complex care and research. Thus for the university hospital and academia in general, some feel that the new payment rules are certain to lead to a decrease in the number of university hospitals and may provide convenient means for shrinking the nation's entire medical education system. The per-case prospective payment concept has some merit because it rewards economy rather than consumption. The use of the diagnosis related groups on the other hand does not appear to be an accurate measure of the use of hospital resources, and university hospitals suffer most in systems using them.

An examination of the New Jersey experience shows that it is difficult to add new programs and equipment that are necessary to carry out teaching and research. Once rates have been established for any given period it may be impossible to change them, and when a system reaches maturity and in a sense all practical efficiencies are realized, new programs can be added only at the expense of existing ones. Unfortunately, experimentation and the unique concerns of university hospitals seldom bear fruit immediately. It would be tempting to assume this danger is averted when costs of the academic institution are passed on to the consumer under the prospective payment system. In this system, different hospitals are paid different prices according to their teaching status, presumably for an identical service. In the current price comparative area the university hospital can be expected to become only the last resort for care. Academic institutions face a dilemma. If they maintain the high and all-inclusive prices they need, they will be too expensive for consumers with a choice. If they do not maintain these high prices, then they lack the support for their special roles. Unfortunately, we do not know the true cost of patient care when separated from the cost of other services of the uni-

versity hospital, that is, education, research, and technology transfer. Who should pay for these costs? The answer, obviously, will determine the future of American medical education. It is surely true that if academic institutions continue to be funded through patient care income, the university hospital will of necessity become less comprehensive and more specialized. Like any other hospital, they will identify "product lines" of greatest profit to them, that is, the types of care they can render competitively. Other cases will be cared for elsewhere. The size and nature of the house staff will be determined by the service needs of the community rather than the dictums from the medical schools.

One academic institution, The Johns Hopkins Hospital, 10 years ago began an operation with a management structure designed to control expenditures by placing responsibility for cost in the hands of physicians. Operating responsibilities were shifted to the clinical departments. In this structure, the larger hospital in effect became a holding company for a series of specialty hospitals referred to as functional units. Each functional unit is headed by a physician-in-chief who is also the chairman of that department in the school of medicine. Reporting to this chief are a nursing director and an administrator. The three function as a management team and are accountable for all direct cost associated with the operation of the unit including services acquired from other departments, such as laboratory medicine and radiology. This has been effective in involving physicians in budgeting and budget management, and the process apparently is moving forward to allow decentralization of both revenue and expense budgeting. This approach recognizes that decisions to bring patients into the hospitals, to recommend diagnostic studies, to prescribe treatment, and to discharge patients generate the majority of hospital expenses. This decentralized management gives the responsibility for these decisions to those who make them, the physicians.

Relman[14] has raised the question "Are teaching hospitals worth the extra cost?" He points out that the average cost per adjusted admission in the more than 300 major teaching hospitals was approximately twice that in nonteaching institutions. When hospitals were paid whatever they charged, this difference in cost was no problem. However, with Medicare switching to a prospective payment system based on fixed payments according to diagnosis related groups, teaching hospitals must cut their cost if they wish to survive. Under the present system, there is no distinction between a patient in a given diagnosis related group who is treated in a nonteaching hospital and one who is treated in a teaching hospital. However, the indirect cost of the house staff and other direct and indirect costs of teaching and research are dealt with through an added calculation by a formula based on the number of house officers per bed. There is no recognition of the cost of free care in teaching hospitals under this system. It is clear, as pointed out by Relman, the special needs of the teaching hospitals will no longer be "automatically accommodated by a passive reimbursement system." Teaching hospitals must therefore convince the government and other third-party payers that their unique and expensive blend of education, research, and tertiary care is worth supporting. Relman concludes, "To survive, they, the university hospitals will need astute leadership and co-operation from the medical staffs."

What has been the reaction to these changes in health care delivery? Public acceptance of large service organizations has always been controversial. Medical care, the most personal of services, is undergoing a change to corporate status as we have noted. For example, almost one third of long-term renal dialysis is provided by one corporation. In addition to the personal nature of this service, the life and death aspects are often only remotely a matter of foreknowledge. If care is urgent, patients go to emergency rooms or tertiary care centers and very frequently cannot name their physician. Patients often say "I had my surgery at the Mayo Clinic or the Massachusetts General," but no physician's name is recalled. Apparently, confidence at times does reside in an institution as well as the individual physician.

If these medical service organizations are acceptable to the public, what effect will this have on the medical profession? Obviously, the part of the profession most susceptible to change is the pool of new medical graduates who apparently are more than adequate in number and thus face greater competition. The trend to medical practice grouping is accelerating so that by the end of the 1990s the majority of the physicians may be so well organized that the independent practitioner will be in the minority. It is suggested that as these groups serve larger populations, they may have multiple service sites and in time serve multiple cities. Each site may then be headed by physicians who "have best adapted to the corporate management style and objectives. The physicians who do not conform to the group objectives, schedules and standards feel peer pressure and stagnation of reward. Some cannot accommodate to peer monitoring and leave." This is surely reminiscent of the plight of Dr. Z in my introductory remarks.

Medical education has given very superficial attention to these changes, and it would seem to me that there must be a thorough discussion and understanding of the direction that medical schools, residency programs, and continuing education programs are headed.

Public attitudes about health care costs are interesting. A recent analysis drawn from 15 national public opinion polls conducted between 1981 and 1984 is particularly relevant. These are the facts regarding health care costs. Despite all that is being done to slow growth of health care cost in the United States, that is, enact-

ment of prospective payment for Medicare (diagnostic related groups), increasing enrollment in health maintenance organization groups (now used by 7% of the population), hospital rate setting in 12 states and other changes, the nation's health care expenditures will increase from $322 billion in 1982 to $690 billion in 1990 and to $1.9 trillion by the year 2000, that is, 14% of the gross national product. This means an average increase of $50 billion a year and a doubling of the nation's health spending every 6 years. The impact of this on the $200 billion federal deficit, the potential bankruptcy of Medicare, and the prospect of renewed inflation leading to another recession are seen as major problems by our national leadership. This year the United States will spend about $1500 per person for health care whereas Japan will spend $500, Great Britain $400, France $800, and West Germany $900.

What is the public attitude toward these facts? Briefly stated, the public opinion survey showed that Americans want the problem of rising health care cost addressed, but they are unwilling to support the adoption of any solution that would produce a dramatic change in their own medical care arrangements. The majority of Americans polled are not in favor of giving up their own personal physicians for care delivered by low-cost physician groups. In fact, a major reason why many Americans choose not to join health maintenance organizations is their reluctance to break ties with their family physician. On the other hand, the public seems to be quite enthusiastic about requiring low-income people to use less costly clinics or health maintenance organizations. The message seems to be "reorganize the nation's health care for others, but don't change my own health care arrangements."

What can be done about this situation? Leaf[15] notes that with medical cost now in excess of 10% of the gross national product, health care encroaches on support for other needs. Virtually all thoughtful studies indicate that decisions by physicians determine between 70% and 80% of all personal medical expenses and thus physicians are the key in setting priorities. However, nothing in medical tradition, training, or experience gives them any expertise to act wisely in making these choices. As Leaf has noted, "On the one hand they have the tradition of exclusiveness of the patient physician relationship and the physician's concerns solely for the patient's welfare. On the other hand, society is paying the bill, not the patient. By making medical decisions, especially those involving the use of costly technology, physicians may be torn between serving the interests of the individual and serving those of society. They have responsibilities to both."

Egdall[16] notes that physicians are being compelled by regulatory initiatives and competition from health maintenance organizations and other alternative delivery systems to reexamine traditional practice routines. It is clear that there are dangers associated with unnecessary hospitalization including the development of nosocomial infections and occurrence of iatrogenic events in hospital environments. Hospitalization obviously is the logical target of efforts to maintain the health care dollar because it is the major component of health care cost.

A number of studies have suggested that high surgical rates can be modified by informational and educational programs. One such study showed the effect of outside surveillance on the rate of hysterectomies in Saskatchewan. The proportion of unjustified hysterectomies as defined by an advisary committee was 23.7% at the time of the first review and dropped to 7.8% at the time of the second review 4 years later. There are wide variations in surgical rates and this does not suggest that surgeons with a higher rate of surgical procedures are practicing poor medicine, but it does mean that in a cost-containment environment surgeons who do not fit the consensus criteria may feel some pressure to justify their admitting patterns, given the dangers and expenses of hospitalization.

Egdall[16] suggests that these savings will come at a price, and indeed one of his suggestions certainly will not be welcomed by hospital administrators. He believes that true cost management must involve shrinkage of the hospital system or use of freed-up capacity resulting from improved practice efficiency to accommodate the needs of our aging population rather than creation of additional capacity. This combined with incentives for surgeons to practice more efficient strategies to strengthen the hospital system as occupancy falls will in his view provide the basis for major community cost containment.

I believe that the important issues in medical practice today include not only the impact of the for-profit movement, but also the trend toward abdication of responsibility and what I call the treatment imperative. It is the rule rather than the exception that patient care is associated with divided responsibility. This is true in group practices and even in the daily surgical rounds and follow-up management. One can justify these practices in the name of better patient care, accessibility, and physician alertness. This platoon system is effective in sports in which it is a question of physical stamina and specialized assignments, but the question of continuity of a process or responsibility is not addressed. I believe it is essential particularly in surgical practice for the operating surgeon to assume full responsibility for the procedure. The litigious attitude of our present society attests to the fact that patients are not entirely happy with this division of responsibility and lack of continuity by their personal physician.

The treatment imperative is particularly subtle. It is the direct result of the newer modalities in treatment with expanded use of chemotherapy and experimental

protocols and also the development of the superspecialist. Extra training implies a necessity to practice what has been learned regardless of the quality of life or the economic impact. The very nature of specialization prevents the physician from viewing his or her actions at a distance. Instead we have the dogma of the training programs—a confidence often based on inexperience and the mysterious language of the clinical trial, p values, and prospective randomized, double-blind studies. This patient is not Mrs. Jones, she is F 103. These studies are necessary, but they have taken a life of their own and frequently obscure obvious medical facts and certainly ethics.

If academia is to survive in this changing medical environment, colleges must emphasize broad intellectual accomplishments rather than a trade school, biochemical approach. Intellectual curiosity, familiarity with the spoken and written word, and yes, manners are important. Medical school deans and admission committees must revise their criteria for admission to include those candidates with a genuine interest in people and patient care in addition to their intellectual achievements as measured by the Medical College Admission Test and their grades. Specialty boards must take a careful look at what they have accomplished. Has the superspecialist become the intellectual automaton acting only on the basis of what he is trained to do for the patient? It is important for us to remember that the pelvic surgeon to women must not be the ambidextrous product of a technical assembly line programmed for increasingly narrow medical skills and intellectual curiosity.

Earlier, I discussed the social conditions of a hundred years ago, the oversupply of physicians, and the increasing dichotomy between the hospital-based physician and the family practitioner. The present drive toward superspecialization, divided responsibility, and the rapid growth of for-profit medical facilities may dissuade the public from any long-range approaches to the problem of rising health care costs including dramatic medical breakthroughs and major positive changes in life-style. They will instead move to short-term solutions—regulation including capital investment controls, state hospital rate setting, and reimbursement controls, competition with the restructuring of health insurance benefits, and the development of alternative health plans and preferred provider organizations that will severely penalize academic institutions and tertiary centers leading to the demise of academic medicine.

None of us has all the answers to control increasing health cost and in particular, hospital care. It is clear that some medical benefits will have to be withheld, at least for some patients. Given this possibility, who will decide which patient is entitled to treatment and what criteria will be used to make this choice? This limitation of medical resources places physicians in an awkward situation. Neither our training nor our long cherished code of ethics prepare us to make such decisions in economic terms.

I began this essay by discussing the plight of physicians at the turn of the century. It is interesting that in a survey of the health care system in Britain, philanthropy continues to play an important role in selected fields of health care, particularly the donation of expensive medical equipment such as the computerized tomographic scanners and the like. Also because of dissatisfaction on the part of the private practitioner, the national health service hospitals retain a few beds for paying patients and the system permits its consultants to have part-time private practice. It appears that we have come full circle and perhaps the lessons of the past will lead us toward a solution of this fiscal and ethical dilemma. We must not be committed to one or another proposal as the *only* solution to rising health care costs. Our allegiance to a single solution stands in the way of a national effort to solve this problem. We must build a consensus around a cost-containment program that realistically reflects public opinion and satisfies the professional and ethical concerns of physicians. Failure to act promptly and decisively surely will hasten the demise of academia—the foundation on which effective health care must stand.

REFERENCES

1. Warner RA. Dispensaries: management and development. New York: Macmillan, 1918.
2. Vietor AC. The abuse of medical charity, the passing of the charity hospital and the dispensary. Boston Med Surg J 1899;140:417-22.
3. Gay GW. Abuse of medical charity. Boston Med J 1905;15:295-305.
4. Knapp CP. Contract medical work and fees. Bull Am Acad Med 1895;2:86-8.
5. Contract practice. JAMA 1911;57:145-6.
6. Burnum JL. The unfortunate case of Dr. Z: How to succeed in medical practice in 1984. N Engl J Med 1984;310:729-30.
7. Schnabel TG. Is medicine still an art? N Engl J Med 1983;309:1258-61.
8. Grouse LD. Has the machine become the physician? [Editorial]. JAMA 1983;250:1891.
9. Crawshaw R. JAMA. Technical zeal or therapeutic purpose—how to decide? JAMA 1983;250:1857-9.
10. Ginzberg E. The monetarization of medical care. N Engl J Med 1984;310:1162-5.
11. Relman AS. The new medical industrial complex. N Engl J Med 1980;303:963-70.
12. Relman AS. Investor-owned hospitals and health care costs. N Engl J Med 1983;309:370-2.
13. Cunningham RM. Sounding boards: entrepreneurialism in medicine. N Engl J Med 1983;309:1313-4.
14. Relman AS. Are teaching hospitals worth the extra cost? N Engl J Med 1984;310:1256-7.
15. Leaf A. Sounding board: the doctor's dilemma—and society's too. N Engl J Med 1984;310:719-20.
16. Egdahl RH. Ways for surgeons to increase the efficiency of the use of hospitals. N Engl J Med 1983;309:1184-7.

Effect of low-dose aspirin on angiotensin II pressor response in human pregnancy

Luis Sanchez-Ramos, M.D., Mary Jo O'Sullivan, M.D., and Jose Garrido-Calderon, M.D.
Miami, Florida, and Santo Domingo, Dominican Republic

Recent clinical studies have reported a significant reduction in the incidence of pregnancy-induced hypertension after the ingestion of low-dose aspirin. The effect of 80 mg of acetylsalicylic acid on vascular sensitivity to exogenous angiotensin II (Hypertensin, Ciba-Geigy Limited, Basel, Switzerland) was examined in 13 normotensive pregnant patients. The effective pressor dose before treatment (17.4 ± 2.2 ng/kg/min) (mean ± SE) was significantly less ($p < 0.001$) than that after treatment (35.1 ± 4.2 ng/kg/min). Low-dose aspirin therapy resulted in an enhancement of the pregnancy-acquired refractoriness to angiotensin II. It can be speculated that prostglandin synthetase inhibitors at a low dose may alter the thromboxane A_2/prostacyclin ratio in favor of the latter. (Am J Obstet Gynecol 1987;156:193-4.)

Key words: Angiotensin II sensitivity, preeclampsia, aspirin

During normal human pregnancy, as early as the first trimester, there is marked refractoriness to the pressor effects of infused angiotensin II. In women destined to develop pregnancy-induced hypertension, this refractoriness is lost as early as 22 weeks' gestation.[1, 2]

Why refractoriness to angiotensin II occurs is unknown. It has been suggested that this refractoriness is prostaglandin mediated. Studies with the use of moderate doses of prostaglandin synthetase inhibitors before and after angiotensin II infusion in pregnant women have shown a significant decrease in the effective pressor dose of angiotensin II, demonstrating increased angiotensin II sensitivity in all patients studied.[3, 4]

Low-dose aspirin is capable of inhibiting thromboxane A_2 production with minimal effect on the synthesis of prostacyclin, thus possibly restoring a critical thromboxane-prostacyclin balance.[5]

Recent studies have shown that aspirin may prevent pregnancy-induced hypertension by inhibiting thromboxane A_2 production.[6, 7]

The purpose of this study was to pursue whether low-dose aspirin would affect angiotensin II sensitivity.

Material and methods

Thirteen primigravid women participated in the study. They were all 28 to 34 weeks' gestation and had no evidence of hypertension or of cardiac or renal disease. The patients were recruited from the antenatal clinics at the Maternity Hospital of the Dominican Institute for Social Services in Santo Domingo, Dominican Republic. Informed consent was obtained from all patients after the study was formally approved by the Maternity Hospital's Committee on Human Investigation.

The patients were placed in a quiet room in the left lateral position for the duration of the study. Blood pressure measurements were obtained every 5 minutes until a stable diastolic pressure was achieved for 15 minutes, by means of a standard sphygmomanometer on the left upper arm. The first and fifth sounds of Korotkoff were recorded as the systolic and diastolic pressure, respectively. An infusion of angiotensin II (Hypertensin, Ciba-Geigy Limited, Basel, Switzerland) was administered with an infusion pump (Model No. 1001, MED Fusion Systems, Inc., Norcross, Georgia). The infusion contained 50 μg of angiotensin II in 50 ml of 5% dextrose in water (1 μg/ml) and was started at a rate of 4 ng/kg/min. The dose was increased by small increments every 5 minutes until the minimum amount of angiotensin II that increased the diastolic pressure by 20 mm Hg was reached (the effective pressor dose).

When the effective pressor dose was established and maintained for 2 to 3 minutes, the angiotensin infusion was discontinued and the diastolic blood pressure was allowed to return to its baseline value. Confirmation of the effective pressor dose was established by restarting the angiotensin II infusion at the same rate that had previously elicited a 20 mm Hg increase in the diastolic pressure.

After the validity of the pretreatment effective pressor dose of angiotensin II was established, the infusion was discontinued and each patient was given 80 mg of

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Miami, and the Department of Obstetrics and Gynecology, Maternity Hospital-Dominican Institute for Social Services (IDSS).
Received for publication June 3, 1986; accepted July 22, 1986.
Reprint requests: Luis Sanchez-Ramos, M.D., Department of Obstetrics and Gynecology, University Hospital of Jacksonville, 655 West Eighth St., Jacksonville, FL 32209.

Table I. Effect of low-dose aspirin (80 mg) on the angiotensin II pressor dose in 13 pregnant subjects

Patient No.	*Gestational age (wk)*	*Angiotensin II pressor dose (ng/kg/min)*	
		Before aspirin	*After aspirin*
1	34	8.4	19.6
2	30	7.0	14.0
3	28	21.2	54.0
4	32	13.6	29.2
5	29	19.6	36.4
6	28	8.4	31.6
7	31	22.8	39.2
8	34	20.4	38.2
9	33	11.2	19.6
10	29	19.6	28.0
11	32	33.6	59.6
12	29	28.0	61.6
13	31	12.6	25.3
Mean		17.2	35.1
SEM		±2.2	±4.2
		$p < 0.001$	

aspirin. Two hours later, the angiotensin II infusion was restarted to establish the effective pressor dose after low-dose aspirin in the same manner as before the treatment.

Results

Thirteen primigravid women, 28 to 34 weeks' gestation, were studied before and after the administration of low-dose aspirin (80 mg). The effective pressor dose of angiotensin II in each of these patients was greater after treatment than before. The mean ± SEM pretreatment value of the group was 17.4 ± 2.2 ng/kg/min and increased to 35.1 ± 4.2 ng/kg/min, which is significantly greater than pretreatment levels ($p < 0.001$). Each patient studied demonstrated a blunted pressor response with low-dose aspirin (Table I).

Comment

The results of the present study demonstrate a significant decrease in angiotensin pressor responsiveness (increased refractoriness) 2 hours after oral administration of 80 mg of aspirin to apparently normal primigravid women in the third trimester of pregnancy. This is in contrast to previous reports that found enhanced sensitivity to angiotensin II after higher doses of prostaglandin synthetase inhibitors.[3, 4]

This discrepancy may be due to selective inhibition of platelet-derived thromboxane A_2 by low-dose aspirin in contradistinction to the inhibition of both thromboxane A_2 and prostacyclin with higher doses of prostaglandin synthetase inhibitors. Aspirin, ≥40 mg/day, inhibits both vascular wall prostacyclin production and platelet thromboxane production, but the effect of aspirin on thromboxane A_2 is greater.[5, 9]

Alterations in the production of prostaglandins may play an important role in the pathogenesis of pregnancy-induced hypertension. An imbalance in opposing actions of thromboxane A_2 and prostacyclin on platelet aggregation and vessel tone may be responsible for some of the clinical and laboratory manifestations of preeclampsia. Thromboxane A_2 is a potent vasoconstrictor and stimulator of platelet aggregation by the placenta and platelets, whereas prostacyclin is a vasodilating agent with opposing effects on platelet activity.[7] Enhanced sensitivity to angiotensin II, causing decreased refractoriness, may be the first sign of such an imbalance, which is tilted in favor of thromboxane A_2. Unfortunately serum and/or urinary levels of the principal metabolites of thromboxane and prostacyclin were not determined in the clinical studies cited or in any of the reports, including ours, on the effects of prostaglandin synthetase inhibitors on the pressor response to angiotensin II. We are currently undertaking such a project.

This study serves to strengthen the theory that pregnancy-related refractoriness to angiotensin II may be mediated by the action of prostaglandins and suggests the possibility that low-dose aspirin may affect prostaglandin imbalances and thus contribute to the reduction of the sensitivity to vasoactive agents characteristic of preeclampsia.

REFERENCES

1. Gant NF, Daley GL, Chand S, Whalley PJ, MacDonald PC. A study of angiotensin II pressor response throughout pregnancy. J Clin Invest 1973;52:262-9.
2. Oney T, Kaulhausen H. The value of the angiotensin sensitivity test in the early diagnosis of hypertensive disorders in pregnancy. Am J Obstet Gynecol 1982;142:17-20.
3. Everett RB, Worley RJ, MacDonald PC, Gant NF. Effect of prostaglandin synthetase inhibitors on pressor response to angiotensin II in human pregnancy. J Clin Endocrinol Metab 1978;46:1007-10.
4. Jaspers WJ, De Jone PA, Mulder AW. Angiotensin II sensitivity and prostaglandin synthetase inhibition in pregnancy. Eur J Obstet Gynecol Reprod Biol 1981;11:379-84.
5. Hanley SP, Cockbill SR, Bevan J, Hepinstall S. Differential inhibition by low dose aspirin of human venous prostacyclin synthesis and platelet thromboxane synthesis. Lancet 1981;2:969-71.
6. Beaufils M, Coniomoni R, Uzan S, Colan JC. Prevention of pre-eclampsia by early antiplatelet therapy. Lancet 1985;1:840-2.
7. Wallenburg HC, Makovitz JW, Dekker GA, Rotmans P. Low dose aspirin prevents pregnancy induced hypertension and pre-eclampsia in angiotensin sensitive primigravida. Lancet 1986;1:1-3.
8. Walsh WS. Pre-eclampsia: an imbalance in placental prostacyclin and thromboxane production. Am J Obstet Gynecol 1985;152:335-40.
9. Preston FE, Whipps S, Jackson CA, French AJ, Wyld PJ, Stoddard CJ. Inhibition of prostacyclin and platelet thromboxane A_2 after low dose aspirin. N Engl J Med 1981; 302:76-9.

Deoxyribonucleic acid hybridization analysis for the detection of urogenital *Chlamydia trachomatis* infections in women

Chia C. Pao, Ph.D., Shyh-Shyan Lin, M.S., Tsyr-En Yang, M.S., Yung-Kuei Soong, M.D., Pai-Shun Lee, R.N., and Jung-Yaw Lin, Ph.D.

Taipei, Taiwan, Republic of China

The presence of *Chlamydia trachomatis*–related deoxyribonucleic acid sequences in endocervical specimens of 317 women was analyzed by deoxyribonucleic acid hybridization techniques with deoxyribonucleic acid from *C. trachomatis* used as probes. Samples from 56 of 172 high-risk patients (32.6%) and 16 of 145 low-risk patients (11.0%) contained *C. trachomatis*–related deoxyribonucleic acid sequences. Direct detection of chlamydial antigen with enzyme-linked immunoassay on the same patients yielded positive rates of 26.3% and 7.3% for the high- and low-risk patients, respectively. *C. trachomatis* culture confirmed 86.3% of deoxyribonucleic acid–positive results and 84.0% of antigen-positive results. The overall sensitivities of chlamydial deoxyribonucleic acid and antigen assays were 91.7% and 68.8%, respectively, whereas the specificities were 95.3% and 94.7%. Results also suggested that the test of the *C. trachomatis* deoxyribonucleic acid correlated better with the female urogenital chlamydial infections than did the antigen test of *C. trachomatis*. The combined results of higher sensitivity in detecting the microorganism and better correlation with disease activity may make the deoxyribonucleic acid hybridization test a useful tool for the early and accurate diagnosis of *C. trachomatis* infections in female patients. (AM J OBSTET GYNECOL 1987;156:195-9.)

Key words: *Chlamydia trachomatis*, female urogenital infection, deoxyribonucleic acid hybridization, deoxyribonucleic acid probe

Infections caused by *Chlamydia trachomatis*, now recognized as the most prevalent of all sexually transmitted diseases, are very damaging and can result in a wide range of diseases including trachoma and lymphogranuloma venereum.[1-3] Men, women, and infants are all affected, but women bear a disproportionately heavy burden because of their increased risk for other adverse reproductive consequences. In women, *C. trachomatis* may cause mucopurulent cervicitis and urethral syndrome in the lower urogenital tract and endometritis and salpingitis in the upper urogenital tract. These infections can also result in sterility and other complications during pregnancy.[3-5] Although chlamydial infections can now be effectively treated with antibiotics, one of the most difficult aspects of controlling chlamydial infection in women is that unrecognized and misdiagnosed chlamydial infections in women are common. It has been suggested that a substantial portion of women infected with chlamydiae may be asymptomatic.[2, 3]

From the Departments of Biochemistry and Obstetrics and Gynecology, Chang Gung Memorial Hospital, and the Department of Biochemistry, National Taiwan University School of Medicine.

This work was supported entirely by Research Grant MRP-131 awarded to C. C. P. from Chang Gung Memorial Hospital, Taipei, Taiwan, Republic of China.

Received for publication May 30, 1986; accepted August 14, 1986.

Reprint requests: Professor Chia C. Pao, Department of Biochemistry, Chang Gung Memorial Hospital, 199, Tun Hwa North Road, Taipei, Taiwan 10560, Republic of China.

Chlamydiae possess a unique development cycle. Although they are now classified as bacteria, they share many properties with viruses. Chlamydiae are obligatory intracellular parasites and depend on the energy supply of the host cells to grow.[6] For this reason, culture of chlamydiae is laborious and requires tissue culture techniques and facilities. Time and expenses are increased further when subculturing is needed, which is quite often. Therefore the culture method has not become routine procedure in the laboratory diagnosis of chlamydial infection. Direct cytologic staining is available, but it has only limited applications because of its lacking of specificity and it requires a considerable amount of experience if false positive and false negative results are to be avoided. In recent years, antigen detection methods, such as direct-smear immunofluorescence staining tests[7] and enzyme-linked immunoassays,[8] have improved rapidly; these offer an alternative to the conventional culture and cytologic staining methods and open the door to further investigation of the broad spectrum of diseases caused by this microorganism.

In the following text, we report the use of deoxyribonucleic acid (DNA) fragments from the human biovar strain of *C. trachomatis* in a DNA hybridization test on clinical specimens to determine its value as a reliable culture-independent diagnostic test in the detection of chlamydial infection. The DNA hybridization test was found to have compared favorably with the conventional culture and antigen results.

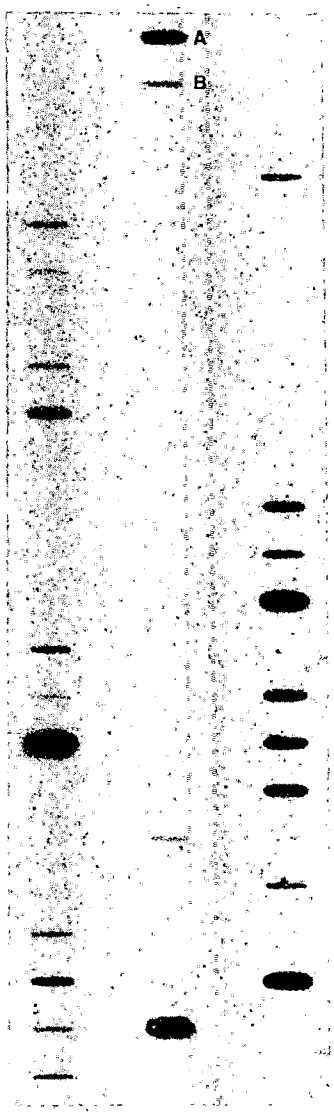


Fig. 1. Autoradiographs of the chlamydial DNA slot blot hybridization test of endocervical samples. Shown here are the DNA results of 60 patients participating in this study. Specimens were treated and handled according to the procedures outlined in Material and methods. The actual size of each slot was 0.75 by 8 mm, or 6 mm^2. Slots *A* and *B* were positive controls of 20 and 2 pg of chlamydial DNA, respectively.

Material and methods

Study protocol. Individuals visiting the outpatient clinic of the Department of Obstetrics and Gynecology of the Chang Gung Memorial Hospital in Taipei, Taiwan, were recruited for this study. A total of 172 patients who had signs or complaints of one or more of the following symptoms were included in the high-risk group: primary infertility (65 patients), urethritis or urethral syndrome (29 patients), cervicitis (10 patients), cervical erosion (nine patients), pelvic inflammatory disease (53 patients), tubal disorders (15 patients; 12 confirmed by laparoscopy and three suspected), pr vious ectopic pregnancy (six patients), recurrent or h bitual abortions (24 patients), and previous stillbirth c neonatal death (three patients). Another 145 patien who were matched in age and in marital and socioec nomic status and who visited the clinic for reasons othe than those listed above were selected as the lov risk group. Informed consent was obtained from a patients.

Laboratory procedures. Two endocervical and/c urethral swabs were collected with the STD-EZE swa (Abbott Laboratories, Inc., North Chicago, Illinois). E tracts from the swabs were pooled and divided in three equal portions for chlamydial DNA, antigen, an culture analysis. Blood samples were also drawn f antichlamydial antibody determination. Chlamydial a tigen was determined by the enzyme-linked immun assay kit Chlamydiazyme, commercially obtained fro Abbott.[8] Antichlamydial antibody was assayed wit an enzyme-linked immunoassay kit obtained fro Exdevelop Pharma, Täby, Sweden. Chlamydial DNA i the endocervical swab was determined by a modifie DNA:DNA hybridization technique that we have pr viously used for the quantitation of hepatitis type virus DNA,[9] which was based on methods initially d scribed by others.[10] Briefly, a purified cloned chlamydi plasmid DNA of 6.7 kilobases, pLGV125,[11] consistin only of the chlamydial plasmid genome was used probe. This DNA was originally cloned by Dr. St ven H. Larsen of Indiana University School of Medicir and was kindly made available to us. Alternatively, cloned DNA coded for the major outer membrane pr tein of *C. trachomatis* serovar L2, pFEN207,[12] obtaine from Dr. F. E. Nano of Rocky Mountain Laboratori was used as a probe. The DNA probe was labeled wit [alpha-^{32}P]-deoxycytidine 5′-triphosphate by nick tran lation of the purified chlamydial DNA to approximate 1×10^8 cpm per microgram of DNA. DNA from e docervical material was extracted with phenol and d natured with 40 μl of sodium hydroxide, 2 mol/L, an sodium chloride, 2 mol/L, for 10 minutes at room ten perature. Then 75 μl of Tris hydrochloride, 1 mol/l pH 7.4, and sodium chloride, 4 mol/L, was added neutralize the reaction mixture. Each specimen w deposited on a 0.45 μm pore size nitrocellulose men brane filter sheet through wells of a microsample fi tration manifold under vacuum (BA85 and Min fold II, both from Schleicher and Schuell, Inc., Keen New Hampshire). After loading, the nitrocellulose filt was gently removed from the manifold under conti ued vacuum, blotted, air dried, and baked at 70° C f 2 hours in vacuo. DNA hybridization was performe at 65° C with about 2×10^7 cpm of the labeled DN used as probe for each filter of 63 by 228 mm in siz After prehybridization and hybridization, the filte

Table I. Prevalence of chlamydial DNA, antigen, culture, and antichlamydial antibody in high- and low-risk women

	High risk		*Low risk*		*Overall*	
Type of assay	*n*	%	*n*	%	*n*	%
Chlamydia DNA	56/172	32.6*	16/145	11.0*	72/317	22.7
Chlamydia antigen	35/133	26.3†	9/124	7.3†	44/257	17.1
Chlamydia culture	39/106	36.8‡	9/92	9.8‡	48/198	24.2
Antichlamydia antibody	29/152	19.1§	18/117	15.4§	47/269	17.5

Percentages represent prevalence, which is equal to number of patients with positive results per number tested in each assay and patient group.

*$\chi^2 = 20.68$, $p < 0.001$.

†$\chi^2 = 16.35$, $p < 0.001$.

‡$\chi^2 = 19.55$, $p < 0.001$.

§$\chi^2 = 0.61$, $p < 0.5$.

Table II. Sensitivity and specificity of *Chlamydia trachmatis* DNA and antigen assays

		*Sensitivity**		*Specificity†*	
Type of assay	*Risk group*	%	*n*	%	*n*
DNA assay	High	89.7	35/39	97.0	65/67
	Low	100.0	9/9	94.0	78/83
	Overall	91.7‡	44/48	95.3	143/150
Antigen assay	High	74.4	29/39	95.5	64/67
	Low	44.4	4/9	94.0	78/83
	Overall	68.8‡	33/48	94.7	142/150

*Sensitivity = number of samples that were DNA and culture positive or antigen and culture positive per number of samples that were culture positive.

†Specificity = number of samples that were DNA and culture negative or antigen and culture negative per number of samples that were culture negative.

‡$\chi^2 = 7.94$, $p < 0.01$.

were washed sequentially in 2X SSC, 0.1% sodium dodecyl sulfate, 30 minutes twice at room temperature (1X SSC = sodium citrate, 15 mmol/L, and sodium chloride, 150 mmol/L, pH 7.0); 0.1X SSC, 0.1% sodium dodecyl sulfate, also 30 minutes twice at room temperature; and 0.1X SSC, 0.5% sodium dodecyl sulfate, 30 minutes twice at 65° C. Filters were then air dried and autoradiographed for 24 to 48 hours. After autoradiography, the filters were cut out and counted in a liquid scintillation counter. The amount of chlamydial DNA in the swab samples was calculated according to the chromic phosphate P 32 counts obtained from a series of dilutions of purified chlamydial DNA prepared from a stock of known concentration and hybridized to the probe DNA on the same filters. The sensitivity of detection of our method was determined to be approximately 1.0 pg of chlamydial DNA. For the purpose of analyzing the results, a DNA concentration readout of ≥2.0 pg was interpreted as a positive result in this study (Fig. 1).

Culture for chlamydia was performed with the use of cycloheximide-treated McCoy cell monolayers as described in detail elsewhere.[13]

Results

The incidence of endocervical chlamydia with the DNA, antigen, and culture techniques as well as the finding of serum antichlamydial antibody is shown in Table I. The incidences displayed by the high-risk patients were significantly higher than those from the low-risk group in three of four categories, excepting antichlamydial antibody, where the difference between the high- and low-risk groups was not significant. The overall positive rates for DNA and culture were close, and both were significantly higher than the positive rate for chlamydial antigen (Table I). The two DNA probes, pLGV125 and pFEN207, gave essentially the same results. The mean age of the infected participants, based on either the DNA, antigen, or culture findings, did not differ significantly from the mean age of those who were not infected.

A comparison of sensitivity and specificity of the DNA and antigen tests is presented in Table II. The overall sensitivity was 91.7% for the DNA test and 68.8% for the antigen test ($p < 0.01$). On the other hand, the DNA and antigen tests showed equally high degrees of specificity. The overall specificity was 95.3%

for the DNA test and 94.7% for the antigen test. On the basis of an average incidence of 22.7% for the DNA test and 17.1% for the antigen test, the predictive values of positive results were 86.3% and 84.0% for the DNA and antigen tests, respectively. For negative results, the predictive values were 97.3% and 90.4%.

For 198 patients with a complete set of DNA, antigen, and culture test results available, the discordance between culture results and those of antigen and DNA was analyzed. (1) Of the 150 culture-negative patients, eight were found to be antigen positive and seven were DNA positive. The chlamydial DNA concentrations in the endocervical specimens of these seven patients were below 20% of the mean DNA concentrations of all patients tested, and four of these seven patients responded to treatment. The endocervical chlamydial DNA tests became negative 2 weeks after the patients were treated with Vibramycin. If these four patients were infected, the resolved sensitivity of the DNA test would be 92.3% instead of 91.7% (Table II). (2) Among the 48 culture-positive patients, as many as 15 were not recognized by the chlamydial antigen test, and four were negative for the DNA test. Of these 15 culture-positive/antigen-negative patients, 14 were found to have chlamydia on DNA testing. (3) Fourteen of 21 patients who were antigen negative but had chlamydial DNA were confirmed to have chlamydial infection on the basis of culture results. On the other hand, only three of 11 patients who were antigen positive but with no DNA in the endocervical specimens had confirmation of chlamydiae by culture.

Comment

Several lines of evidence indicated that the higher sensitivity of the DNA method was accompanied by a better reflection of the actual disease status: (1) Chlamydial DNA sequences could be detected in none of the specimens from the eight patients with false positive antigen results; (2) specimens from 14 of the 15 patients with false negative antigen results (93.3%) contained chlamydial DNA; (3) the respective culture confirmation rates for the patients with chlamydia antigen-negative/DNA-positive and antigen-positive/DNA-negative results were 66.7% (14 of 21) and 27.3% (3 of 11) ($\chi^2 = 4.36$, $p < 0.05$). The possibility of using the DNA probe to detect chlamydiae in clinical specimens such as endocervical materials was further supported by the report that the chlamydial plasmid DNA used here as a probe, pLGV125, had extensive homologous DNA sequences in all 15 *C. trachomatis* serovars.[11] In addition, little if any DNA homology could be detected with bacteria of other genera that were likely to be present in locations where chlamydiae might be found.[14]

The 68.8% overall sensitivity achieved by the enzyme-linked immunoassay (Chlamydiazyme, Abbott) that detects an antigen of *C. trachomatis* was lower than what has been reported by others.[8] We could not rule out the possibility that this discrepancy was due to the presence of a different but very closely related antigen in our patient population. However, this discrepancy did not seem to be related to the reported cross-reactivity between Chlamydiazyme and organisms other than chlamydiae.[15]

Historically, time-consuming and expensive tissue culture techniques were required to definitively diagnose most *C. trachomatis* infections. Despite recent rapid and profound improvements in the antigen and antibody detection systems, these tests are not ideal, mainly because of their sensitivity and their lack of complete correlation with the disease activity. The usefulness of DNA:DNA hybridization has been recognized recently in the identification of a number of microorganisms and is particularly helpful in cases of infectious agents that are traditionally difficult to culture. The DNA hybridization system used here seemed to have higher sensitivity, without compromising specificity, in the detection of chlamydial infection when compared with the commercially available enzyme-linked immunoassays. Assuming a plasmid DNA length of 6.7 kilobases and 10 copies of plasmid molecules per chlamydia genome equivalent,[11] a sensitivity of 2 pg represents a level of detection of about 30,000 chlamydia genome equivalents. When the number of elementary and reticulate bodies in each infected cell is considered, the minimal number of infected cells needed for detection by the DNA probe system will actually be even lower. Such higher sensitivity could offer earlier detection and treatment of subclinical or latent chlamydial infections in women, many of whom were asymptomatic, thus reducing the incidence of serious complications and sequelae. It also opened new doors for the detection of chlamydial infections under other clinical conditions, such as during pregnancy. Chlamydial infection during pregnancy may lead to both serious sequelae in the mother and infections such as pneumonia or eye infection in infants.

In summary, results of the chlamydial DNA hybridization test showed a higher sensitivity than the antigen detection method in the diagnosis of chlamydial infections and correlated highly with the disease activity in our patient population. The results of our study indicated that the DNA hybridization test will contribute significantly to the early and accurate diagnosis of urogenital chlamydial infections in women.

We thank Dr. Delon Wu and Dr. Chau-Hsiung Chang for their interest and encouragement during this study. Appreciation is also expressed to Miss Wen-Ling Yang and Miss Ruey Ken for their technical assistance.

REFERENCES

1. *Chlamydia trachomatis* infections: policy guidelines for prevention and control. MMWR 1985;34(3S):53S-74S.
2. Thompson SE, Washington AE. Epidemiology of sexually transmitted *Chlamydia trachomatis* infections. Epidemiol Rev 1983;5:96.
3. Sweet RL, Schachter J, Landers DV. Chlamydial infections in obstetrics and gynecology. Clin Obstet Gynecol 1983; 26:143.
4. Martin DH, Koutsky L, Eschenbach DA, et al. Prematurity and perinatal mortality in pregnancies complicated by maternal *Chlamydia trachomatis* infections. JAMA 1982;247: 1585.
5. Johannisson G, Lowhagen GB, Lycke E. Genital *Chlamydia trachomatis* infection in women. Obstet Gynecol 1980;56: 671.
6. Schachter J, Caldwell HD. Chlamydiae. Annu Rev Microbiol 1980;34:285.
7. Tam MR, Stamm WE, Handsfield HH, et al. Culture-independent diagnosis of *Chlamydia trachomatis* using monoclonal antibodies. N Engl J Med 1984;310:1146.
8. Howard LV, Coleman PF, England BJ, Herrmann JE. Evaluation of Chlamydiazyme for the detection of genital infections caused by *Chlamydia trachomatis*. J Clin Microbiol 1986;23:329.
9. Liaw YF, Tai DI, Chu CM, et al. Early detection of hepatocellular carcinoma in patients with chronic type B hepatitis. Gastroenterology 1986;90:263.
10. Lieberman HM, LaBrecque DR, Kew MC, Hadziyannis SJ, Shafritz DA. Detection of hepatitis B virus DNA directly in human serum by a simplified molecular hybridization test: comparison to HBeAg/Anti-HBe status in HBsAg carriers. Hepatology 1983;3:285.
11. Hyypia T, Larsen SH, Stahlberg T, Terho P. Analysis and detection of Chlamydial DNA. J Gen Microbiol 1984; 130:3159.
12. Nano FE, Caldwell HD. Expression of the chlamydial genus-specific lipopolysaccharide epitope in *Escherichia coli*. Science 1985;228:742.
13. Clyde WA, Kenny GE, Schachter J. Laboratory diagnosis of chlamydial and mycoplasmal infections. In: Drew WL, ed. Cumulative techniques and procedures in clinical microbiology, Cumitech 19. Washington DC: American Society for Microbiology, 1984.
14. Kingsbury DT, Weiss E. Lack of deoxyribonucleic acid homology between species of the genus *Chlamydia*. J Bacteriol 1968;96:1421.
15. Saikku P, Puolakkainen M, Leinonen M, Nurminen M, Nissinen A. Cross-reactivity between Chlamydiazyme and acinetobacter strains. N Engl J Med 1986;314:922.

Divergent effects of two low-dose oral contraceptives on sex hormone–binding globulin and free testosterone

Claudia Jung-Hoffmann, M.D., and Herbert Kuhl, Ph.D.

Frankfurt am Main, Federal Republic of Germany

The effect of a triphasic combination of ethinyl estradiol and levonorgestrel on the serum concentrations of total testosterone, free testosterone, and sex hormone–binding globulin as measured directly by radioimmunoassay and on the binding capacity of sex hormone–binding globulin was compared with that of a preparation containing ethinyl estradiol and desogestrel. Blood samples were taken on days 6, 11, 21, and 28 of a control cycle, the third cycle of treatment with either ethinyl estradiol–levonorgestrel or ethinyl estradiol–desogestrel (11 volunteers each), the third cycle of a 3-month washout period, and the third treatment cycle after crossover change of the preparations. There was a significant reduction in total testosterone by 16% during treatment with both preparations. Ethinyl estradiol–desogestrel increased the concentration (+175%) and bindig capacity (+330%) of sex hormone–binding globulin to a much greater extent than with ethinyl estradiol–levonorgestrel (+92% and +160%). Contrary to this, a significant suppression of non–protein-bound testosterone by 35% was found during treatment with both oral contraceptives. The results demonstrate that an excessive elevation of the levels of sex hormone–binding globulin above the normal range does not cause a corresponding suppression of free testosterone. It is assumed that the decrease in the apparent binding affinity of high sex hormone–binding globulin concentrations to testosterone may be due to protein-protein interactions. (Am J Obstet Gynecol 1987;156:199-203.)

Key words: Low-dose oral contraceptives, sex hormone–binding globulin, testosterone, free testosterone

From the Division of Gynecological Endocrinology, Department of Obstetrics and Gynecology, J. W. Goethe University.
Received for publication March 3, 1986; revised July 1, 1986; accepted August 14, 1986.

Reprint requests: Dr. Herbert Kuhl, Division of Gynecological Endocrinology, Department of Obstetrics and Gynecology, J. W. Goethe University, Theodor-Stern-Kai 7, D-6000 Frankfurt am Main 70, Federal Republic of Germany.

Non–protein-bound testosterone, which is assumed to be the biologically active testosterone fraction in serum, is a function of total testosterone and sex hormone–binding globulin (SHBG) levels. It was found that the calculation of the ratio of testosterone to SHBG ("free androgen index"), when compared with total testosterone, provides a more reliable parameter in assessing androgen status in hirsute women.[1,2] Treatment with ethinyl estradiol was found to increase SHBG concentrations in a dose-dependent manner while androgens and nortestosterone-derived progestogens exert a suppressive effect. Therefore, treatment of hirsute women with suitably composed oral contraceptives was recommended for the improvement of androgenetic manifestations.[3]

It has been suggested that the extent of elevation of SHBG correlates negatively with the free testosterone level and positively with the clinical effect.[4] Consequently, it has been claimed that oral contraceptives that cause an excessive increase in serum SHBG should be the first choice for women with symptoms of hyperandrogenism of ovarian origin. The free androgen index, however, is a derived value, and there is some doubt whether this holds true.

In contrast to desogestrel, levonorgestrel has been shown to counteract in a dose-dependent way the stimulatory effect of ethinyl estradiol on hepatic SHBG production.[5-7] As we are capable of measuring directly the serum concentrations of free testosterone, total testosterone, and SHBG by means of radioimmunoassays, we investigated the effect on these parameters of a triphasic combination of ethinyl estradiol and levonorgestrel and a preparation containing ethinyl estradiol and desogestrel, which is known to increase SHBG levels excessively.

Material and methods

Subjects. Twenty-two healthy volunteers, aged 24 to 35 years, participated in the study. Informed consent was obtained. The women had not used hormonal contraceptives for at least 3 months before the study and were not receiving pharmacologic therapy that would interfere with the study. An internal gynecologic examination, including a Papanicolaou smear, was carried out before the selection of the subjects. During a control cycle preceding the study, ovulation was confirmed to have taken place by the determination of the serum progesterone level (>3 ng/ml) on days 19 to 22 of the cycle.

Preparations. The combined triphasic preparation contained 30 μg of ethinyl estradiol plus 50 μg of levonorgestrel (6 days), 40 μg of ethinyl estradiol plus 75 μg of levonorgestrel (5 days), and 30 μg of ethinyl estradiol plus 125 μg of levonorgestrel (10 days) (Triquilar, Schering AG, Berlin, Federal Republic of Ger many). The other combined preparation contained 3(μg of ethinyl estradiol plus 150 μg of desogestrel (2 days) (Marvelon, Organon GmbH, Oberschleissheim Federal Republic of Germany).

Study design. Single blood samples were obtaine(on days 6, 11, 21, and 2E of the control cycle precedin the first treatment cycle (Control 1). Eleven wome were randomly assigned to use at first the triphasi ethinyl estradiol–levonorgestrel preparation and an other 11 volunteers were designated to use the ethiny estradiol–desogestrel preparation, each for 3 months The randomization procedure was carried out b means of a random permutations table, the odd num bers being assigned to the first group and the eve numbers to the other. The first treatment perio was followed by a washout period of 3 months (Con trol 2). Thereafter the contraceptive preparations wer changed in a crossover fashion; that is, the first grou was now assigned to use the ethinyl estradiol–desoges trel contraceptive for 3 months, and the second group the triphasic ethinyl estradiol–levonorgestrel prepa ration. Single blood samples were also taken at 8:00 AM on days 6, 11, 21, and 28 of the third treatment cycle the third washout cycle, and the third treatment cycl after the crossover of the preparations.

Laboratory methods. After centrifugation of th blood samples, the serum was frozen and stored a −20° C until laboratory analyses. Serum concentration of testosterone were measured by means of a "direct radioimmunoassay kit supplied by Sorin Biomedic (Hamburg, Federal Republic of Germany). The assa range was 0.87 to 34.7 nmol/L; the mean intra-assa coefficient of variation, 7.2%; and the mean inter assay coefficient of variation, 8.1%. Cross-reaction were as follows: 5α-dihydrotestosterone, 7.2%; andro stenedione, 0.8%; dehydroepiandrosterone, 0.036% 5α-androstan-3α,17β-diol, 0.03%; and other steroids <0.02%.

Serum concentrations of SHBG were determine with the use of a "liquid-phase" immunoradiometri assay kit supplied by Farmos Diagnostica (Oulunsalc Finland). The assay range was 6.25 to 100 nmol/L; th mean intra-assay coefficient of variation, 3.2%; and th mean interassay coefficient of variation, 5%. No huma serum protein is known to cross react with the double antibody combination used in the assay. The bindin capacities of SHBG in serum were measured accord ing to the method described by Hammond and Läh teenmäki[8] with tritiated 5α-dihydrotestosterone used a marker. The mean intra-assay coefficient of variatio was 6.6% and the mean interassay coefficient of vari ation was 8.1%. The free testosterone fraction was de termined by means of a solid-phase radioimmunoassa

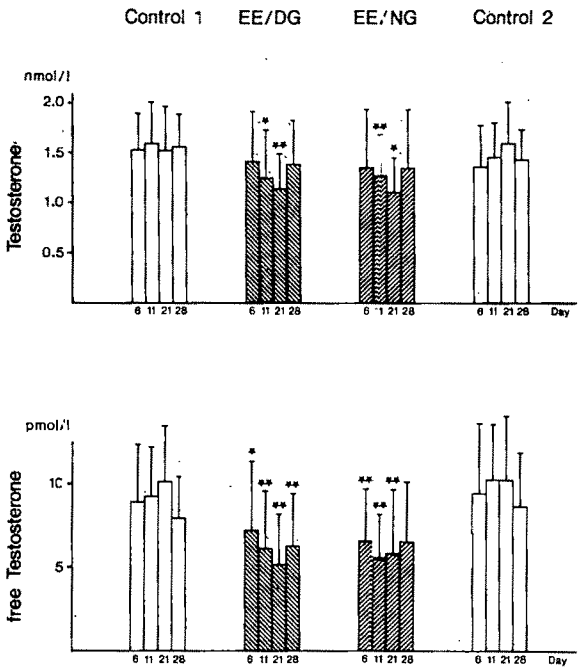


Fig. 1. The effect of ethinyl estradiol–desogestrel *(EE/DG)* and ethinyl estradiol–levonorgestrel *(EE/NG)* on the serum concentrations of total and free testosterone (mean ± SD). ⋆ = Different from control group, $p < 0.05$. ⋆⋆ = Different from control group, $p < 0.01$.

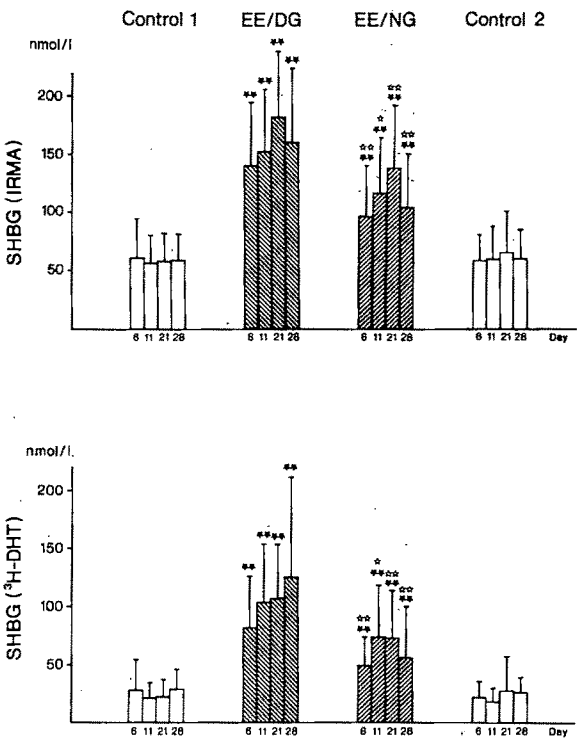


Fig. 2. The effect of ethinyl estradiol–desogestrel *(EE/DG)* and of ethinyl estradiol–levonorgestrel *(EE/NG)* on the serum concentration of SHBG (by immunoradiometric assay, *IRMA*) and on the binding capacity of SHBG (by tritiated dihydrotestosterone, *³H-DHT*) (mean ± SD). ⋆⋆ = Different from control group, $p < 0.01$. ⋆ = Different from *EE/DG*, $p < 0.05$. ⋆⋆ = Different from *EE/DG*, $p < 0.01$.

(Coat-A-Count Free Testosterone) supplied by Diagnostic Products Corporation (Los Angeles, California) with the use of an antiserum with an affinity for testosterone slightly less than that of SHBG. The assay range was 5.2 to 173 pmol/L; the mean intra-assay coefficient of variation, 3.8%; and the mean interassay coefficient of variation, 4.2%. Cross-reactions included: 5α-dihydrotestosterone, 0.041%; androstenedione, 0.01%; 5α-androstan-3β,17β-diol, 0.005%; Δ^5-androstene-3β-17β-diol, 0.007%; 5α-androstan-3,17-dione, 0.001%. There was no effect of an increase in the albumin level by 25%.

Statistical analysis. Data were analyzed by univariate analysis of variance by application of a three-factor experiment with repeated measurements on two-factor design.[9, 10] The volunteers were assigned to two groups depending on the sequence of administration of the drugs. Within-group factors were treatment comprising the four levels of control, ethinyl estradiol–levonorgestrel preparation, washout, and ethinyl estradiol–desogestrel preparation and the days of the cycle (days 6, 11, 21, and 28). A posteriori contrasts were tested by Scheffé's procedure with use of the appropriate error terms as described in detail by Glaser.[10] The level of significance was set at 0.05. The calculations were carried out on an Apple IIe computer.

Wilcoxon's matched-pairs signed-ranks test was used for the evaluation of the significance of differences between mean values on the respective days. The inverse correlation between the concentrations of SHBG and free testosterone was evaluated by calculation of the regression curves (second order) after transformation to the regression line: log (concentration SHBG) = A + B × X (concentration free testosterone), with the use of a computer program.

Results

During treatment with both ethinyl estradiol–levonorgestrel and the ethinyl estradiol–desogestrel preparation there was a significant ($p < 0.05$) reduction in total testosterone by 16% to average values between 1.11 and 1.42 nmol/L (Fig. 1).

The SHBG concentration as determined by immunoradiometric assay was significantly increased on an average from 60.2 ± 27.2 to 115.5 ± 48.5 nmol/L (+92%) by the triphasic preparation and to 165.9 ± 57.8 nmol/L (+175%) by the ethinyl estradiol–desogestrel combination (Fig. 2). The difference between both preparations was significant. The SHBG binding capacity, as determined by means of the tritiated 5α-di-

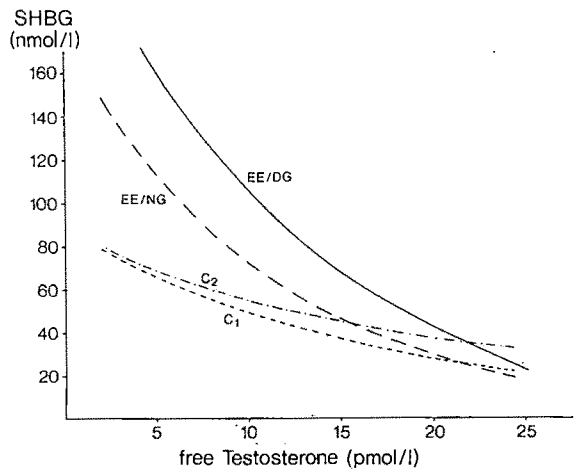


Fig. 3. Regression curves (second order) between the serum concentrations of SHBG and free testosterone (*EE/NG* = ethinyl estradiol–levonorgestrel; *EE/DG* = ethinyl estradiol–desogestrel; C_1 = control cycle; C_2 = washout cycle).

hydrotestosterone binding assay, rose from 24.3 ± 17.1 to 63.2 ± 37.5 nmol/L (+160%) during treatment with the triphasic ethinyl estradiol–levonorgestrel and to 105.0 ± 56.4 nmol/L (+330%) by the ethinyl estradiol–desogestrel combination (Fig. 2). The differences between the values of the control and treatment cycles and those between both preparations were significant.

When the free testosterone concentrations were measured directly, a significant suppression by 35% from an average of 9.37 ± 3.36 to 6.10 ± 3.35 pmol/L was found during treatment with both low-dose oral contraceptives (Fig. 1). Contrary to the effects on the concentrations and binding capacities of SHBG, there was no difference between the ethinyl estradiol–levonorgestrel and the ethinyl estradiol–desogestrel combination.

The discrepancy between the SHBG and the free testosterone levels became obvious when the regression curves (second order) were calculated from the corresponding values of the control, ethinyl estradiol–levonorgestrel, and ethinyl estradiol–desogestrel groups (Fig. 3). There was only a poor inverse correlation between the concentrations of SHBG and the free testosterone fraction, particularly at very high SHBG levels.

Comment

The present results show that treatment of women with the triphasic ethinyl estradiol–levonorgestrel preparation causes an elevation of SHBG levels by approximately 100%, while serum SHBG increased threefold to fourfold during the intake of the ethinyl estra- low-dose contraceptives suppressed the serum concen tration of total testosterone by 16%. Similar finding had been reported by several authors.[5-7, 11] The evalu ation of the free testosterone fraction by means of mea suring the SHBG binding capacity or by calculating th ratio of testosterone to SHBG, as carried out by mos investigators, would result in free testosterone level during treatment with ethinyl estradiol–levonorgestre that were twice those with ethinyl estradiol–desogestrel These calculated values, however, do not represen physiologic conditions, as some other steroid-bindin proteins are neglected. Those studies that had use more appropriate methods for the determination o free testosterone levels[5, 7, 11] did not compare the tw low-dose preparations.

Our results clearly demonstrate that the free testos terone fraction, as determined directly, is suppressed b both oral contraceptives to the same extent (−35%), al though the SHBG levels are much higher during treat ment with the ethinyl estradiol–desogestrel combina tion. When comparing the effects of two different con traceptive preparations Gaspard et al.[5] found that fre testosterone was decreased by 45% by both the triphasi ethinyl estradiol–levonorgestrel combination and by monophasic combination of 50 μg of ethinyl estradic and 250 μg of levonorgestrel, while SHBG was elevate (+85%) only by the former preparation. Mathur et al.[1] reported that apparent free testosterone concentra tions correlate with total testosterone levels but not wit SHBG. There are some indications that other bindin proteins, particularly albumin, may be involved to a higher degree than generally assumed. It was pointe out by Westphal[13] that the concept of the unbound ste roid hormone, as usually evaluated, is an oversimplifi cation. The hormone-protein interaction provides a dy namic binding system, involving association and disso ciation, and there are no static phases of bound an unbound steroid. According to the law of mass action the concentration of testosterone that is actually avail able is dependent not only on the binding constants bu also on the concentrations of the binding components.

It has been shown that the uptake of testosterone b target organs is considerably inhibited by both albumi and SHBG.[14] The uptake was in direct proportion t the free testosterone concentrations, which were simila in the presence of 90 μmol/L of human serum albumi and 90 nmol/L of SHBG. Moreover, it was demon strated that the effect of SHBG or albumin on the leve of free testosterone is more conspicuous at lower con centrations, while an increase above the normal rang of SHBG results only in minor alterations of unboun steroid. When the SHBG level was increased to mor than 50 nmol/L, the uptake of testosterone by the tissu

This was corroborated by studies of the effect of norgestrel on the binding of estradiol and testosterone to SHBG. When the serum level of SHBG was <50 nmol/L, the percent binding of the steroids was directly related to the SHBG concentration. However, when SHBG exceeded 50 nmol/L, the fractions of free steroids were independent of the serum level of SHBG but paralleled the total concentrations of testosterone and estradiol.[15] This corresponds to the present findings, as total and free testosterone levels were similarly depressed by both low-dose oral contraceptives, while the SHBG levels were raised excessively above the normal range to a different extent.

The reason for the decrease in the relative binding capacity of very high SHBG levels is unknown. It was, however, observed that the association constant of the albumin-testosterone complex decreases with increasing albumin concentrations, possibly because of protein-protein interactions.[13] The apparent binding affinity of cortisol to corticosteroid-binding globulin was also found to decrease with rising concentrations of the protein.[16] It is conceivable that similar interactions may affect the binding affinity of SHBG at high concentrations. It was demonstrated by Petra et al.[17] that the metabolic clearance rate of testosterone is inversely related to SHBG. The effect, however, is small, and is related to the distribution of testosterone bound between SHBG and albumin. There appears to exist an optimal upper level of SHBG in serum, and no matter how much more SHBG is circulating there will be little change in the metabolic clearance rate.

In women with androgenetic manifestations, an elevation of low SHBG levels may be followed by a decrease in free testosterone and an improvement of clinical symptoms. An excessive increase of SHBG above the normal range, however, does not seem to result in a further reduction of free testosterone, provided that total testosterone is normal. It appears to be of more clinical significance for women with hyperandrogenism that elevated levels of total testosterone are reduced by oral contraceptives. It is known that a pronounced increase in serum SHBG during treatment with hormonal contraceptives reflects a preponderance of the effect of the estrogen component. With respect to other possible side effects, a moderate change of estrogen-sensitive biochemical parameters seems to be more advantageous.

REFERENCES

1. Badawy SZA, Cittadino R, Marshall L. Testosterone: testosterone estradiol binding globulin ratio in evaluating hirsute women. Int J Fertil 1982;27:166.
2. Carter GD, Holland SM, Alaghband-Zadeh J, Rayman G, Dorrington-Ward P, Wise PH. Investigation of hirsutism: testosterone is not enough. Ann Clin Biochem 1983; 20:262.
3. Raj SG, Raj MHG, Talbert LM, Sloan CS, Hicks B. Normalization of testosterone levels using a low estrogen-containing oral contraceptive in women with polycystic ovary syndrome. Obstet Gynecol 1982;60:15.
4. Cunningham SK, Loughlin T, Culliton M, McKenna TJ. Plasma sex hormone-binding globulin and androgen levels in the management of hirsute patients. Acta Endocrinol 1983;104:365.
5. Gaspard UJ, Dubois M, Gillain D, Franchimont P, Duvivier J. Ovarian function is effectively inhibited by a low-dose triphasic oral contraceptive containing ethinylestradiol and levonorgestrel. Contraception 1984;29:305.
6. Cullberg G, Dovre P-A, Lindstedt G, Steffensen K. On the use of plasma proteins as indicators of the metabolic effects of combined oral contraceptives. Acta Obstet Gynecol Scand 1982;111(suppl):47.
7. Hammond GL, Langley MS, Robinson PA, Nummi S, Lund L. Serum steroid binding protein concentrations, distribution of progestogens, and bioavailability of testosterone during treatment with contraceptives containing desogestrel or levonorgestrel. Fertil Steril 1984;42:44.
8. Hammond GL, Lähteenmäki PLA. A versatile method for the determination of serum cortisol binding globulin and sex hormone binding globulin binding capacities. Clin Chim Acta 1983;132:101.
9. Kirk RE. Experimental design: procedures for the behavioral sciences. Belmont, California: Brooks/Cole, 1968.
10. Glaser WR. Varianzanalyse. Stuttgart: Gustav Fischer Verlag, 1978.
11. Vermeulen A, Thiery M. Metabolic effects of the triphasic oral contraceptive Trigynon. Contraception 1982;26:505.
12. Mathur RS, Moody LO, Landgrebe S, Williamson HO. Plasma androgens and sex-hormone-binding globulin in the evaluation of hirsute females. Fertil Steril 1981;35:29.
13. Westphal U. Steroid-protein interaction II. Berlin: Springer-Verlag, 1985.
14. Mercier-Bodard C, Marchut M, Perrot M, Picard M-T, Baulieu E-E, Robel P. Influence of purified plasma proteins on testosterone uptake and metabolism by normal and hyperplastic human prostate in "constant-flow organ culture." J Clin Endocrinol Metab 1976;43:374.
15. Stumpf PG, Nakamura RM, Mishell DR. Changes in physiologically free circulating estradiol and testosterone during exposure to levonorgestrel. J Clin Endocrinol Metab 1981;52:138.
16. Mueller UW, Potter JM. Binding of cortisol to human albumin and serum: the effect of protein concentration. Biochem Pharmacol 1981;30:727.
17. Petra PH, Stanczyk FZ, Senear DF, et al. Current status of the molecular structure and function of the plasma sex steroid-binding protein (SBP). J Steroid Biochem 1983; 19:699.

Source of prenatal care and infant birth weight: The case of a North Carolina county

Paul A. Buescher, Ph.D., Clinton Smith, M.D., M.P.H., Joseph L. Holliday, M.D., M.P.H., and Ronald H. Levine, M.D., M.P.H.

Raleigh, North Carolina, Nashville, Tennessee, and Greensboro, North Carolina

The impact of a comprehensive prenatal care program on the birth weights of infants born to low-income women is assessed. Women receiving care through the prenatal program of a large county public health department were compared to pregnant Medicaid-eligible women in the same county, who received prenatal care primarily from private-practice physicians. The percentage of low birth weight was 8.3 for the health department women compared with 19.3 for the Medicaid women. After differences between the two groups in race, marital status, participation in the Special Supplemental Food Program for Women, Infants, and Children (WIC), quantity of prenatal care, and other risk factors were statistically controlled, the chance of a Medicaid woman having a low-weight birth was still more than twice as great ($p = 0.007$). A case-management approach and greater use of services ancillary to basic obstetric medical care appear to contribute to the better birth weight outcomes in the health department. (Am J Obstet Gynecol 1987; 156:204-10.)

Key words: Prenatal care, low birth weight, Medicaid

It has been convincingly demonstrated that low birth weight greatly increases the risk of subsequent infant morbidity and mortality.[1, 2] Low birth weight also frequently results in higher medical care costs.[3] Thus the prevention of low birth weight has become a major concern of the health care community. Some of the more important factors shown to influence the risk of low birth weight are race, age, marital status, socioeconomic level, multiple birth, and prenatal care.[4]

The impact of prenatal care on birth weight has received considerable attention, in part because, among the major risk factors, prenatal care is probably the most amenable to change through deliberate health programs. Most studies of prenatal care and birth weight have looked at the quantity and timing of prenatal care, such as number of visits or month prenatal care began. More refined measures such as the Kessner Index consider number of visits and month care began in relation to length of gestation.[5] Such quantitative measures of care may be easily calculated from birth certificate data if the required components are recorded. Receiving much less attention, however, is how the scope and quality of prenatal care affect birth weight. The recent report by the Institute of Medicine on preventing low birth weight points to the need for more research on the content of prenatal care.[4]

A comprehensive prenatal care program for low-income women in 13 California counties resulted in significantly lower rates of low birth weight for these women in comparison with those in a matched control group of Medicaid women.[6] The present study looks at the effect of comprehensive prenatal care on the birth weights of infants born to low-income women in a North Carolina county. Women receiving prenatal care at the Guilford County Public Health Department are compared with Guilford County women on Medicaid, who received prenatal care mainly from private-practice physicians. It was hypothesized that the comprehensiveness of the health department's prenatal care program has a substantial positive impact on birth weights. If true, this would have important implications for provision of prenatal care to low-income women, where the problem of low birth weight is severe.

Material and methods

Guilford County, North Carolina, is a metropolitan area with a population of approximately 325,000. The county has a large and active public health department, with around 340 full-time-equivalent staff positions. Prenatal care is provided at two sites, in the cities of Greensboro and High Point. This project was initiated as a means of evaluating a health department program in terms of outcome (birth weight), rather than a "process" measure such as number of clients served. The

From the Division of Health Services, North Carolina Department of Human Resources, the Maternal and Child Health Section, Tennessee Department of Health and Environment, and the Guilford County Public Health Department.

Supported in part by National Institutes of Health Contract No. NO1-HD-4-2845.

Received for publication May 2, 1986; revised July 21, 1986; accepted August 14, 1986.

Reprint requests: Paul A. Buescher, Ph.D., State Center for Health Statistics, Division of Health Services, P. O. Box 2091, Raleigh, NC 27602.

prenatal care program was chosen because it is one of the larger health department programs and because its outcome could be assessed with information from birth certificates. The results of this study turned out to have implications beyond the effectiveness of this particular health department program.

The first step was to indicate which of the 4037 Guilford County resident birth certificates for 1984 belonged to women who were in the prenatal care program of the health department. A list of the 426 women who received prenatal care at the health department and had a live birth in 1984 was provided by the health department and contained the name of each woman, the date of delivery, the date of her birth, and race. Computer and subsequent manual matching according to these variables resulted in successful identification of 396 birth certificates for babies born to health department women (a matching rate of 93%). Thus the "case" group became Guilford County residents who received prenatal care in this health department.

Guilford County women on Medicaid were chosen as a comparison group, primarily because they could be identified from Medicaid paid claims data. In Guilford County there is an agreement between the health department and the obstetricians that women who are eligible for Medicaid and then become pregnant will receive prenatal care through the private-practice physicians. The health department does not have the capacity to serve all indigent women in the county and its first responsibility is to the women with no insurance. Thus pregnant Medicaid-eligible women in this county are referred to the obstetricians' office-based practices by the health department or by the Department of Social Services. This type of arrangement may not exist in some counties in North Carolina, and indeed many Medicaid-eligible women in other areas do receive prenatal care from public health departments. It so happens, however, that in Guilford County the comparison of "health department" and "Medicaid" birth outcomes becomes essentially a comparison of public and private sources of care.

Like the women receiving prenatal care at the health department, the Medicaid-eligible women are predominantly poor and nonwhite. The income level for Medicaid eligibility is only one third to one half the poverty level in North Carolina, so this is a very low-income group of women that may differ in some ways from the health department women. A woman receiving prenatal care at the health department must be a resident of Guilford County and certified as having a family income of less than the poverty level. Potential problems of comparability between these two groups are considered later in this report.

In Guilford County most of the active obstetricians rotate hospital duty and thus the same physicians handle the deliveries of both the health department and Medicaid women. Therefore any differences in low birth weight between the two groups may be attributed mainly to differences in prenatal care rather than to differences in hospital management or intrapartum care.

Birth certificates for Medicaid women were identified by matching to Medicaid paid claims, with the use of name and age of mother. All 1984 inpatient hospital Medicaid claims for Guilford County residents with an ICD-9-CM diagnosis of 640-676 (conditions related to pregnancy and delivery) were selected, resulting in an unduplicated count of 556 women. Ninety percent (or 500) of these claims were successfully matched to birth certificates. It was expected that some of these claims would not match since some Medicaid hospital stays are for obstetric complications with no live birth occurring at the time. The matching indicated that 138 women were in both groups, that is, they received prenatal care at the health department and Medicaid paid for the hospital delivery. The health department and private-practice physicians have agreed that if a woman begins prenatal care at the health department and subsequently becomes eligible for Medicaid, she will complete her prenatal care at the health department for the sake of continuity, although at the time of delivery Medicaid would be available to pay for hospital charges. These women are counted in the health department group here since that is where prenatal care was received.

Besides the indicator of whether the woman received prenatal care in the health department or from a private-practice physician through Medicaid, most of the information used in this analysis is from the birth certificate: race, age, marital status, education, single or multiple birth, previous fetal death or death after live birth, birth weight, and quantity of prenatal care. The Kessner Index was used as an indicator of the quantitative adequacy of prenatal care. It considers number of visits and month care began in relation to length of gestation (see Table I for definition).* For purposes of this analysis, quantity of prenatal care was dichoto-

*Completed weeks of gestation, calculated from date of birth and date of last menstrual period on the birth certificate, was the only variable from the birth certificate used in this analysis that had more than a few missing values. Where only day of the month of date of last menstrual period was missing, the fifteenth was imputed. If calculated weeks of gestation was <18 or >48 for these live births, gestation was recoded to unknown. The calculated weeks of gestation was then compared with birth weight and, where the two were clearly incompatible, weeks of gestation was recoded to unknown. These procedures, plus the records with month or year of date of last menstrual period missing, resulted in about 2% of the births having unknown weeks of gestation and therefore unknown Kessner Index.

Table I. Criteria for adequacy-of-care index levels*

Adequacy of care	*Trimester of first prenatal visit*		*Gestation (wk)*		*No. of prenatal visits*
Adequate	First (1-3 mo)	and	18-21	and	≥3
			22-25	and	≥4
			26-29	and	≥5
			30-31	and	≥6
			32-33	and	≥7
			34-35	and	≥8
			≥36	and	≥9
Inadequate	Third (7-9 mo)	or	18-21	and	0 or not stated
			22-29	and	≤1 or not stated
			30-31	and	≤2 or not stated
			32-33	and	≤3 or not stated
			≥34	and	≤4 or not stated
Intermediate	All other combinations				

The index was treated as missing if a component was missing or if calculated weeks of gestation for the live birth was <18 or >48.

*Adapted from Kessner DM, Singer J, Kalk CE, Schlesinger ER. Infant death: an analysis by maternal risk and health care In: Contrasts in health status. Washington DC: Institute of Medicine, National Academy of Sciences, 1973, vol 1.

Table II. Live births in 1984 to residents of Guilford County, North Carolina, by source of prenatal care, birth weight, and selected risk factors for low birth weight

	Source of prenatal care		
	Public health department	*Private practice physician through Medicaid*	*All other*
No. of births	396	362	3279
<2500 gm (%)	8.3	19.3	7.1
Nonwhite (%)	65.4	81.5	25.2
Unmarried (%)	60.6	86.7	12.5
Age under 18 (%)	17.2	13.5	4.2
Education <12 yr (%)	47.8	45.6	14.9
Multiple birth (%)	2.8	3.9	1.9
Previous fetal death or death after live birth (%)	20.2	24.3	20.3
Not on WIC (%)	22.5	55.5	94.4
Prenatal care not adequate in quantity (Kessner Index) (%)	44.0	38.2	14.2

mized into adequate ("adequate" index) or not adequate ("intermediate" or "inadequate" index).

In addition to the information from the birth certificate, an indicator of whether the woman participated in the Special Supplemental Food Program for Women, Infants, and Children (WIC) before delivery was added. This was done by selecting Guilford County residents from a file of WIC women having a live birth in 1984 and matching on name and date of delivery. Out of 687 WIC records for Guilford County residents, 650 or 95% were matched with a 1984 Guilford County birth certificate.

The main analytical tool used here to address the question of the effect of source of prenatal care on birth weight among low-income women was logistic regression. An indicator (1 or 0) of whether the birth was low weight (<2500 gm) was used as the outcome measure. In assessing the effect of source of prenatal care on birth weight, it was necessary to control for other important influences on birth weight, for example, race, marital status, and quantity of prenatal care. The usual regression techniques are not appropriate when the dependent variable is dichotomous. Using logistic regression allows assessment of how source of prenatal care affects the odds of having a low-weight birth, after other important influences are controlled.

Results

Table II describes 1984 live births to Guilford County residents in terms of source of prenatal care, birth weight, and selected risk factors for low birth weight. Health department women and Medicaid women accounted for 9.8% and 9.0%, respectively, of 1984 Guilford county resident births. Women in these two groups

were much more likely than other Guilford County women to be nonwhite, unmarried, under age 18, of low education, and on WIC and to have had prenatal care that was not adequate in quantity. In a comparison of the Medicaid and health department groups, the Medicaid women were considerably more likely to be nonwhite, unmarried, and not on WIC. Since most Medicaid women qualified in 1984 through the Aid to Families with Dependent Children (AFDC) program, which mainly provides support to single-parent households, the very high proportion of the women that were unmarried is understandable.

The difference between the Medicaid and health department women in percentage of births that were low weight, 19.3 versus 8.3, is dramatic. (This difference is statistically significant at much less than $p = 0.001$.) Perhaps most surprising is that the percentage of low-weight births among the health department women was only slightly higher than that for the "all other" group shown in Table II, in spite of this being a "high-risk" group. The health department women had better birth weight outcomes than the Medicaid women even though the quantity of prenatal care (in terms of number of visits and timing of care) received by these women was, on the average, lower than that for the Medicaid group. Further examination of the data revealed that the somewhat lower quantity of care received by the health department women was entirely a function of starting prenatal care after the first trimester, rather than having fewer visits once care was begun.

In order to conclude more definitely that the nature of the prenatal care provided in the health department contributes to this difference in low birth weight, it is necessary to control for differences between the two groups in other risk factors for low birth weight. Table III considers the results of a logistic regression model with low birth weight as the dependent variable. Only the health department and Medicaid births are included here ($N = 758$). It can be seen that even after differences between the health department and Medicaid women in race, marital status, WIC, quantity of prenatal care, and the other measured risk factors were controlled, a woman receiving prenatal care from a private-practice physician through Medicaid was on the average more than twice as likely to have a low-weight birth ($p = 0.007$). Reasons for this strong effect will be considered in the following section.

The strongest effect in the model is that of multiple birth. A woman with a nonsingleton birth was more than 18 times as likely to have a low-weight baby than a woman with a singleton birth, independent of all other factors. Not being on WIC independently increased the chances of having a low-weight birth by 60% among the low-income women in this study. This is further corroboration of the effectiveness of the WIC program in preventing low-weight births. The independent effect of race on low birth weight shown here is not highly significant ($p = 0.18$), but it should be remembered that these two groups of women are predominantly nonwhite, with much less variation in race than would be found among all the births of a county or state. Other characteristics with a strong, independent effect on the probability of a low-weight birth are being unmarried, age under 18, and having had a previous fetal death or death after a live birth.

Table III. Estimated relative odds of low birth weight with presence of selected risk factors* (1984 Guilford County Health Department and Medicaid births, N = 758)

Risk factor	*Relative odds*	*p*
Nonwhite	1.58	0.18
Unmarried	1.80	0.11
Age under 18	1.92	0.06
Education <12 yr	.86	.57
Multiple birth	18.60	<.0001
Previous fetal death or death after live birth	1.94	0.01
Not on WIC	1.60	0.06
Prenatal care not adequate (quantity)	1.37	0.18
Medicaid provider of care (versus Health Dept.)	2.08	0.007

*These odds are derived from a logistic regression model and refer to the relative likelihood of a woman with the characteristic delivery of a low-weight baby compared with a woman without the characteristic. These are independent effects, that is, after the effects of all other variables in the model are controlled. The p values refer to the probability that this effect is due to random variation, given the sample size (758) and relative frequency of the variable (1 or 0). The model likelihood ratio χ^2 for testing the joint significance of all variables in the model is 78.82 with 9 degrees of freedom, $p < 0.01$.

Comment

Most evaluations of the effect of prenatal care on birth outcome have looked at the quantity of care in terms of the month of pregnancy in which care began and total number of prenatal visits. Indeed, these are the variables most likely to be recorded on birth certificates, and measures of content or quality are much harder to obtain. In the present study, the health department women actually had a lower quantity of prenatal care, on the average, than the Medicaid women, while having much better birth weight outcomes. Thus, if consideration of prenatal care had been limited to the Kessner Index or other quantitative measures of care, little effect on low birth weight would have been shown among these low-income women. Examining the nature and content of the prenatal care provided by the health department may provide insight concerning this differential.

The Guilford County health department provides a

comprehensive, coordinated system of prenatal care with extensive written protocols. Each woman is evaluated on entry and an individual prenatal care plan is developed. Special provisions are made to screen and educate the women about preventing preterm labor. The highest-risk women are referred to a high-risk maternity clinic at Moses Cone Hospital, a tertiary care center. Health education, counseling, and other health department services are also provided for these women. The women at nutritional risk are referred to the WIC program, which is administered through the health department. This link with WIC is very important, and Table II reveals that nearly 80% of the health department women are on WIC compared with less than half of the Medicaid women. Table III indicates that WIC does have a significant, positive, and independent impact on birth weight in this county. Higher participation in WIC could in fact be considered as one quality of the health department prenatal care program since referral is routine. It appears that the case management and coordination of services provided by the health department are very beneficial and that possibly the Medicaid women in Guilford County are much less likely to benefit from such an approach.

Other studies have also shown the advantages of a comprehensive, coordinated approach to prenatal care, particularly for high-risk, indigent women. Sokol et al.[7] evaluated a comprehensive prenatal care project in Cleveland and found that women on the program had significantly lower perinatal mortality and higher birth weights than women in a comparison group. They concluded that the major difference in prenatal care was in the ancillary services of the project, including nonmedical services such as patient education, home visitation, nutrition assessment and counseling, and social service assessment and intervention. The present study, which goes further to incorporate multivariate adjustments for group differences, strongly supports this perspective. Starfield's review of the literature[8] points to the inability of studies to consistently demonstrate that the amount of prenatal care alone influences the frequency of low birth weight and concludes that the scope and content of care are most important, particularly for high-risk populations. The Institute of Medicine's report on preventing low birth weight indicates that successful prenatal care projects frequently offer a "package" of services including outreach, counseling, and other nonmedical services. Certain populations, especially the poor and very young, "may be better served by public facilities offering a range of services than by physicians in private practice, who traditionally provide only medical care."[4]

One aspect of the Guilford County health department's prenatal care program that may contribute to its better birth weight outcomes, compared with those of the Medicaid women, is the heavy reliance on nurse practitioners for the provision of prenatal care. Sever nurse practitioners in the health department provide the first-line, ongoing care and communication, with specific problems or complications referred to physicians. The Institute of Medicine's report[4] suggests that these providers tend to relate to their patients in a nonauthoritarian manner and to emphasize education support, and patient satisfaction. They are likely to spend more time per visit with their patients than are office-based physicians, and women served by nurse-midwives and practitioners are more likely to keep appointments for prenatal care and to follow specified treatment regimens. In Guilford County, missed visits at the health department are followed by telephone calls, or home visits if necessary.

The follow-up, education, and linkage to other programs found in this health department would probably be less important for a group of highly educated and motivated pregnant women, but they appear to make a great difference for poor pregnant women in Guilford County. Peoples and Siegel[9] concluded that a comprehensive maternity and infant care (MIC) project in North Carolina had the most benefit for those mothers and infants at very high risk. As further indication that the benefits of comprehensive prenatal care are greatest for the highest risk women, the data for this study revealed a strong interaction between source of prenatal care and quantitative adequacy of care. The difference in low birth weight percent between the health department and Medicaid women was predominantly among those women not having an adequate quantity of prenatal care. The logistic regression analysis showed that, with other differences controlled, the risk of low birth weight was 4.5 times greater for the Medicaid women than for the health department women among those not receiving an adequate quantity of prenatal care ($p = 0.001$). For those women whose care was quantitatively adequate, however, the relative risk for the Medicaid women was only 1.2 and not significant ($p = 0.59$). Thus the ancillary services of the health department program appear to be most beneficial among those women who start prenatal care late or have an insufficient number of visits.

Any retrospective study, where random assignment to a "case" and "control" group is not possible, is subject to selection bias. Though the educational levels of the Medicaid and health department women were very similar (Table II), there was no information on income in the present study to use as a control. Since the income cutoff for Medicaid in North Carolina is extremely low it is likely that the Medicaid women in this study were on the average, poorer than the health department women. There could be substantial differences between the health department and Medicaid women on med-

ical complications of pregnancy, social and occupational stress, substance abuse, or other factors that affect birth weight and have not been measured or controlled for in this study. Another possible source of bias is that some women are eligible for Medicaid because they are "medically needy," which usually means that because of previous medical expenses they "spend down" to the Medicaid income level. Thus some of these women could be on Medicaid because of prior health problems. These potential biases could explain part of the much lower birth weights among the Medicaid women, but it is highly unlikely that their control would account for all of the differential. A look at the subset of 138 women who received prenatal care at the health department but qualified for Medicaid before delivery suggests that it is the prenatal care program of the health department, rather than unmeasured differences between the health department and Medicaid women, that accounts for a large part of the birth weight differential. Like the Medicaid women receiving care from private physicians, these 138 women were largely nonwhite and unmarried, yet their percent of low birth weight was 9.4 compared with 19.3 for the other Medicaid women. A logistic regression model for these two groups (N = 500), with the use of the same control variables as in Table III, indicated that the risk of low birth weight was 2.0 times as great for the Medicaid women receiving care in the private sector compared with the 138 women receiving prenatal care at the health department and later being certified for Medicaid ($p = 0.07$).

One possible contribution to the better birth weight outcomes among the health department women is that referrals are made to a high-risk prenatal care clinic at Moses Cone Hospital in Greensboro for women determined to be at risk of preterm labor. Of the 396 women in the health department prenatal care program, about 20% received at least part of their prenatal care at this high-risk hospital clinic. It is not known how many of the Medicaid women were referred to this clinic, although they would be eligible if determined to be at risk for preterm labor. Like referral to WIC, however, the formal linkage to this clinic should be considered a benefit of receiving prenatal care at the health department and not as a "bias" when comparisons are made with the Medicaid group.

A significant finding of this study is that the success of the Guilford County health department's program in preventing low birth weight was almost entirely through reducing preterm births rather than through reducing the incidence of intrauterine growth retardation. The percent of total births that were term and low weight was nearly the same for the health department and Medicaid groups. Much of the positive effect of the health department prenatal care program was through lengthening gestation, a finding that has also been suggested by Showstack et al.[10]

We do not expect that the Guilford County health department is typical in North Carolina in terms of its prenatal care program and it would be desirable to replicate and expand this type of study. In other areas, Medicaid women receiving prenatal care in the health department could be compared with Medicaid women receiving care in private settings, eliminating some of the comparability problems of the present study. It is now possible to match the Medicaid and WIC records to birth certificates on a statewide basis for North Carolina and we hope in time to identify women receiving prenatal care in health departments by means of a statewide automated health services information system. In an expanded study it would be possible, in addition to seeing if the results shown here hold up in other years and geographic areas, to examine the impact of prenatal care on important subcategories of low birth weight, such as births <1500 gm. The numbers were not large enough in the present study to support a more refined analysis of birth weight.

In summary, for women in poverty, a coordinated, comprehensive approach to prenatal care is essential. While visits for obstetric medical care may alone be adequate for women with higher education and income, women with fewer resources need additional services such as health education, counseling, nutritional assessment, and outreach and follow-up. While the present study has been largely a comparison of prenatal care in public and private settings because of the arrangements in Guilford County, the larger issue is the contrast between comprehensive and "traditional" prenatal care. What seems to make the difference are the services that are added to obstetric medical care. Physicians are frequently not able to hire educators, nutritionists, and other providers of ancillary services for their office staffs. What is needed is a coordination of services to assure that low-income women do get access to these needed nonmedical components of prenatal care.

We appreciate the valuable assistance provided by Reva Phillips of the Guilford County Health Department and by J. Michael Bowling of the State Center for Health Statistics.

REFERENCES

1. McCormick MC. The contribution of low birth weight to infant mortality and childhood morbidity. N Engl J Med 1985;312:82-90.
2. Buescher PA, Nugent RR, Levine RH. The impact of low birth weight on North Carolina neonatal mortality 1976-1982. N C Med J 1984;45:437-41.
3. David RJ, Siegel E. Decline in neonatal mortality, 1968 to 1977: better babies or better care? Pediatrics 1983;71: 531-40.

4. Institute of Medicine. Preventing low birthweight. Washington DC: National Academy Press, 1985.
5. Kessner DM, Singer J, Kalk CE, Schlesinger ER. Infant death: an analysis by maternal risk and health care. In: Contrasts in health status. Washington DC: Institute of Medicine, National Academy of Sciences, 1973, vol 1.
6. Korenbrot CC. Risk reduction in pregnancies of low-income women: comprehensive prenatal care through the OB access project. Mobius 1984;4:34-43.
7. Sokol RJ, Woolf RB, Rosen MG, Weingarden K. Risk, antepartum care, and outcome: impact of a maternity and infant care project. Obstet Gynecol 1980;56:150-6.
8. Starfield B. Low birthweight. In: The effectiveness of medical care: validating clinical wisdom. Baltimore: The Johns Hopkins University Press, 1985.
9. Peoples MD, Siegel E. Measuring the impact of programs for mothers and infants on prenatal care and low birth weight: the value of refined analyses. Med Care 1983; 21:586-608.
10. Showstack JA, Budetti PP, Minkler D. Factors associated with birthweight: an exploration of the roles of prenatal care and length of gestation. Am J Public Health 1984; 74:1003-8.

Hairy cell leukemia in pregnancy: A case report

J. Kell Williams, M.D.
Detroit, Michigan

Hairy cell leukemia (leukemic reticuloendotheliosis) is a rare disease. No previously reported cases in pregnancy could be found with the use of available computer-assisted medical literature search programs. The pregnancy was complicated by progressive thrombocytopenia, leukopenia, and anemia that did not improve with delivery. The deterioration was eventually abated by postpartum splenectomy. (Am J Obstet Gynecol 1987;156:210-1.)

Key words: Hairy cell leukemia, pregnancy

Hairy cell leukemia (leukemic reticuloendotheliosis) was first described as an independent clinical and pathologic entity in 1958.[1] It is a rare disease, predominantly of men over the age of 50. No previously reported cases of this form of leukemia in pregnancy could be found with the use of available computer-assisted medical literature search programs.

Case report

D. H., a 34-year-old white woman, gravida 4, para 0-0-3-0, was diagnosed as having hairy cell leukemia at 7 weeks' gestation. Significant past history includes three previous pregnancy losses. The first, which occurred 2 years previously, was a missed abortion at 15 weeks' gestation; the second was an incomplete abortion 1 year previously. Both required a curettage for therapy. Ten months after the second curettage, she was admitted in shock with a ruptured left tubal pregnancy. A salpingectomy was performed. "Low platelets and a low white count" were noted postoperatively and thought to be secondary to multiple transfusions.

From the Department of Gynecology and Obstetrics, Wayne State University School of Medicine.
Received for publication April 11, 1986; revised June 30, 1986; accepted July 10, 1986.
Reprint requests: J. Kell Williams, M.D., Department of Obstetrics and Gynecology, University of South Florida Medical Center, Tampa General Hospital, P. O. Box 1289, Tampa, FL 33601.

The anemia, thrombocytopenia, and leukopenia persisted during the next 4 months. Hematology consultation was obtained. Bone marrow biopsy was reported as showing mononuclear cells with the characteristic cytoplasmic projections pathognomonic of hairy cell leukemia. Also documented was an intrauterine pregnancy of approximately 7 weeks' gestation. Consultation with the maternal-fetal medicine service was obtained.

Initial hematologic studies revealed a hemoglobin of 9.9 gm/dl, a platelet count of 120,000/mm^3, and a white blood cell count of 2100/mm^3. Corticosteroid therapy and/or splenectomy were contraindicated because of the pregnancy. The maternal blood type was A, Rh negative, with a negative antibody screen. Other than the hematologic abnormalities, no other prenatal complications developed. A screening ultrasound examination at 26 weeks' gestation documented normal fetal growth.

The platelet count gradually fell to 70,000/mm^3 at 24 weeks' gestation, 60,000/mm^3 at 28 weeks' gestation, 40,000/mm^3 at 35 weeks' gestation, and eventually 25,000/mm^3 at 37 weeks' gestation. Repeat antibody screen at 32 weeks' gestation had revealed anti-C antibody, with a saline titer of 1:1 and negative Coombs test. The white blood cell count remained in the 2100/mm^3 to 1700/mm^3 range. At 37 weeks, the patient was transfused with 6 units of single-donor platelets. The posttransfusion platelet count was 37,000/mm^3. Nonstress tests, begun at 32 weeks, had been reactive up

until 37 weeks. The nonreactive test at 37 weeks was followed by a suspicious contraction stress test. Induction of labor was begun with oxytocin. After 6 hours of contractions and no signs of fetal distress, the cervix remained undilated while the effacement progressed from 20% to 100%. Six more units of platelets were transfused. The patient had a mild hyperthermic reaction to the transfusion.

After a night's rest, a second day of induction of labor was begun. Two hours into the active phase, persistent late decelerations developed. Dilatation and station did not allow for fetal scalp blood evaluation. A cesarean section was decided on and 1 gm of hydrocortisone was given intravenously. The immediate preoperative hemoglobin was 8.0 gm/dl, the white blood cell count was 1100/mm^3, and the platelet count was 40,000/mm^3. A female infant, weighing 2948 gm, was delivered by low transverse cesarean section with Apgar scores of 7 and 8. Ten more units of platelets and two units of whole blood were transfused intraoperatively. There was no clinical evidence of clotting abnormalities during the operation. The newborn infant had no neonatal complications.

In the recovery room, the patient became short of breath with wet rales throughout all lung fields. Immediate chest x-ray film was consistent with pulmonary edema, likely contributed to by the choice of whole blood for intraoperative transfusion. The patient responded rapidly to the intravenous administration of furosemide (40 mg). Postoperative hemoglobin was 7.9 gm/dl, white blood cell count was 7900/mm^3, and platelet count was 50,000/mm^3. Postpartum antibody screen revealed anti-C, anti-D, anti-CD, and anti-Fya (Duffy). Rh-immune prophylaxis was not indicated.

Moderate thrombocytopenia and leukopenia persisted and a therapeutic splenectomy was performed 5 weeks post partum. Hematologic status improved considerably and has been stable for 18 months.

Comment

Hairy cell leukemia makes up approximately 2% of all cases of leukemia. It is a neoplasia of B lymphocytes, although rare examples of T-lymphocytic forms have been described. There is a strong male predominance with a median age of 55. Presenting symptoms are usually weakness, fatigue, and easy bruising associated with pancytopenia. Classic symptoms of malignant lymphomas (weight loss, night sweats, fever) are present in less than 25% of cases. Approximately 10% of cases are discovered as incidental findings, as in this report. Splenomegaly, not initially noted in this patient, is found in more than 90% of cases at the time of diagnosis. Notably, there is an absence of adenopathy. The course of the disease is usually chronic. The average survival is 6 years, with a 51% actuarial survival at 4 years. The most common cause of death is infection. There are two reported cases of death from massive hemorrhage secondary to splenic rupture and one case of death secondary to hemorrhage into the lung. Splenectomy, with or without chemotherapy, is the treatment of choice with 92% of patients showing improvement of thrombocytopenia.[1,2] More recently, interferon therapy has been shown to have beneficial effects when splenectomy fails to achieve a response.[3]

This report represents a case of hairy cell leukemia in pregnancy. No previous cases could be found by computer-assisted literature search. The diagnosis was made in early pregnancy, before recognition of the gestation. The hematologic status of the patient gradually deteriorated throughout the pregnancy and was not improved with delivery. The deterioration has been abated to date by therapeutic splenectomy.

REFERENCES

1. Bouroncle BA. Leukemic reticuloendotheliosis (hairy cell leukemia). Blood 1979;53:412.
2. Golomb HM, ed. Report of the first international workshop on hairy cell leukemia. Semin Oncol 1984;11:333.
3. Golde DW. Hairy cell leukemia. In: Champlin R, moderator. Chronic leukemias: oncogens, chromosomes, and advances in therapy. Ann Intern Med 1986;104:671.

Sexually transmitted papillomaviral infections

I. The anatomic distribution and pathologic grade of neoplastic lesions associated with different viral types

Richard Reid, M.D., Mitchell Greenberg, M.D., A. Bennett Jenson, M.D., Mutajaba Husain, M.D., Jerry Willett, M.D., Yahya Daoud, M.S., Gary Temple, M.D., C. Robert Stanhope, M.D., Alfred I. Sherman, M.D., Garth D. Phibbs, M.D., and Attila T. Lorincz, Ph.D.

Detroit, Michigan, Washington, D. C., Gaithersburg, Maryland, Rochester, Minnesota, and Toledo, Ohio

Multiple colposcopic biopsy specimens were collected from 160 women, with sampling of principal cervical and vulvar lesions as well as secondary areas of either minor acetowhitening or normal epitheluim. Papillomaviral deoxyribonucleic acid was detected by Southern blot hybridization in 197 (90%) of the 218 principal biopsy specimens and 93 (46%) of 198 secondary biopsy specimens. Although different papillomaviruses were found at different sites in 31 women, only six of 416 specimens contained multiple types within the same sample. Specific viral types were associated with specific disease patterns. Only one of 80 type 6 or 11 infections had a diagnosis greater than cervical intraepithelial neoplasia, grade 2. In contrast, 42 of 48 (90%) biopsy specimens of cervical intraepithelial neoplasia, grade 3, or invasive cancer contained type 16, 18, or 31. Nonetheless, 12 of 124 (10%) cases of condyloma and cervical intraepithelial neoplasia, grade 1, were associated with types 16, 18, and 31 infections. Of 58 women with multicentric disease, 46 had positive hybridizations for both cervical and vulvar lesions (32 showing the same type in both samples and 14 showing different viruses). Differing patterns of papillomavirus-induced disease arise partly from the predilection of specific viral types for certain anatomic sites and partly through variations in host response. Detection of viral deoxyribonucleic acid in 46% of the secondary biopsy specimens suggests that disease expression may represent focal breakdown of host surveillance within a field of latent papillomaviral infection. (AM J OBSTET GYNECOL 1987;156:212-22.)

Key words: Papillomaviruses, sexually transmitted diseases, colposcopy, cervical intraepithelial neoplasia

The papillomaviruses are species-specific deoxyribonucleic acid (DNA) viruses that are widespread in nature, causing characteristic proliferations on epidermal and mucosal surfaces. Papillomaviruses are classified by species and further subdivided into specific types according to nucleotide sequence homology. Most papillomavirus-induced lesions are benign, presenting as either cutaneous warts or mucosal condylomas.[1] However, certain papillomaviral types are oncogenic in vivo and in vitro, and there is now a substantial chain of evidence implicating these agents in the etiology of cervical, vaginal, and vulvar malignancy.[2, 3] The human papillomaviruses comprise three clinicopathologic groups: cutaneotropic viruses found in immunologically normal individuals, cutaneotropic viruses causing epidermodysplasia verruciformis in immunodeficient patients, and mucosotropic viruses infecting the genital, oral, and respiratory mucosae.

Papillomaviruses have a relatively simple structure. All of the viral genetic information is contained within a small circular molecule of double-stranded DNA, having a length of 8000 nucleotide base pairs.[1] The target

From the Departments of Obstetrics and Gynecology and Pathology, Sinai Hospital of Detroit, Department of Pathology, Georgetown University School of Medicine, Bethesda Research Laboratories, Life Technologies, Inc., Division of Gynecologic Surgery, The Mayo Clinic, and Division of Gynecologic Oncology, Medical College of Ohio.

Supported by Grant No. 200-84-0773(P) and Grant No. 200-84-0774, from the Centers for Disease Control, Atlanta, Georgia.

First presented (and awarded the 1986 Presidential Award) at the Seventeenth Annual Meeting of the Society of Gynecologic Oncologists, Palm Springs, California, February 2-5, 1986.

Reprint requests: Richard Reid, M.D., Sinai Hospital of Detroit, Department of Obstetrics and Gynecology, 6767 W. Outer Dr., Detroit, MI 48235.

cells for viral infection are believed to be the basal layer of the affected area of epidermis or mucosa, establishing a viral reservoir that progresses to active expression as progeny cells differentiate during their migration toward the epithelial surface. From the functional viewpoint, the viral genome is divided into "early" and "late" regions. Studies with bovine papillomavirus type 1 indicate that genes from the early region establish and maintain viral control over the infected cell and initiate viral DNA replication. Genes in the late region are involved in capsid protein synthesis and virion assembly.[1-4] Papillomaviruses are unique in that virion production is tied to the cellular events of squamous differentiation and keratinization. Undifferentiated neoplastic cells do not permit completion of the viral life cycle, displaying instead a nonproductive infection with few if any cells progressing to virion assembly. Malignant transformation may occur in such tissues.[5]

Some bovine papillomaviruses have been passaged through many generations of abortive infection in tissue culture, but it has not been possible to induce productive expression or useful cytopathic effect in vitro.[1-4] Viral particles and capsid proteins are sometimes detectable in vivo within benign productive infections, by electron microscopy or immunochemistry. However, viral particles and capsid proteins are rarely present within premalignant or invasive lesions.[5] Fortunately, the availability of radiolabeled viral nucleic acid probes, developed through advances in recombinant DNA technology, has now made it possible to detect papillomaviral genomic sequences within a high proportion of premalignant and malignant tissues. Initial studies showed that condylomas and minor dysplasias generally contained type 6 or 11, while type 16 or 18 was most often detected in high-grade premalignancies or invasive cancers.[6-8] Subsequently, a new papillomavirus called type 31 was detected in about 20% of cervical dysplasias and 5% of cervical cancers obtained from Washington, D. C.[10]

Despite the constancy of these generalizations, current understanding of the natural history of papillomaviral infection in the individual woman is obscured by the markedly different patterns of disease that result from apparently equivalent viral exposures. This study was undertaken in an attempt to clarify some of these basic clinicopathologic paradoxes.

Material and methods

Study design. Type-specific differences in anatomic distribution and pathologic severity of sexually transmitted papillomaviral infections were studied in 160 women seen prospectively between March 1983 and February 1986. Each directed biopsy specimen was collected from an area of uniform appearance, as guided by a previously described colposcopic index.[11]

For the first 85 patients, biopsy specimens were collected systematically from multiple sites within the lower genital tract and analyzed by Southern blot hybridization for types 6, 11, 16, 18, and 31 and unclassified papillomaviruses. Preliminary data analysis showed that there was sufficient information for statistical testing of these secondary biopsy specimens. However, because analysis of the principal biopsy specimens required subdivision into two anatomic sites, five pathologic grades, and seven virologic categories, larger numbers were needed to reach statistical significance. Fiscal constraints dictated that the biopsy protocol in the remaining 75 patients be restricted to sampling areas of obvious abnormality.

Subject material. The study population was drawn mainly from a consecutive series of patients referred to R. R. with either extensive condylomas or varying grades of cervical, vaginal, and vulvar neoplasia. In addition, six samples of invasive cervical cancer were provided by C. R. Stanhope from the Mayo Clinic, Rochester, Minnesota; five samples of invasive cervical or vulvar cancer were provided by A. I. Sherman of Sinai Hospital, Detroit, Michigan, and one case of invasive cancer of the vulva was provided by G. R. Phibbs of the Medical College of Ohio, Toledo, Ohio.

Patients fell within two broad groups: First, 84 women presenting with cervical or vaginal disease, generally detected by Papanicolaou smears (nine with condyloma; 11 with grade 1, 31 with grade 2, and 22 with grade 3 intraepithelial neoplasia; and 11 with invasive cancer). Second, there were 76 women presenting with vulvar disease (53 with vulvar condylomas, 18 with Bowenoid lesions, and five with invasive squamous cancers). Eighteen of the 84 women in the first group had concomitant vulvar lesions, and 40 of the 76 women in the second group had concomitant cervical lesions. Hence the total study material comprised 218 principal lesions collected from 160 women (124 cervical and 94 vulvar lesions).

For the first 85 patients, a systematic series of colposcopic biopsies was performed throughout the lower genital tract, with sampling of (1) the worst area of the presenting lesion, (2) any adjacent area of lesser-grade acetowhitening surrounding the worst area of the presenting lesion, (3) the worst area of any multicentric lesion (a vulvar lesion in a patient presenting with cervical disease, or vice versa), (4) any adjacent area of minor grade acetowhitening at this second site, (5) essentially normal vaginal epithelium (with sampling of any colposcopic "asperities," if present), and (6) in patients with vulvar disease, normal vulvar epithelium at the intended margin of laser ablation. Each biopsy was bisected, half being placed in formalin for paraffin embedding and standard histologic examination, while the other half was frozen at −70° C for virologic anal-

Table I. Detectability of papillomaviral DNA in the 218 principal cervical or vulvar biopsy specimens

Principal cervical biopsy specimens				*Principal vulvar biopsy specimens*			
Diagnosis	*HPV positive*	*Total*	*%*	*Diagnosis*	*HPV positive*	*Total*	*%*
Condyloma or CIN 1	42	53	79	Condyloma or VIN 1	66	71	93
CIN 2	34	38	89	VIN 2	8	8	100
CIN 3 or cervical cancer	31	33	94	VIN 3 or vulvar cancer	13	15	87
Total	107	124	86	Total	87	94	93

HPV = Human papillomaviral infection; CIN = cervical intraepithelial neoplasia; VIN = vulvar intraepithelial neoplasia.

Table II. Type-specific disease associations of the 124 principal cervical biopsy specimens

Worst cervical diagnosis	*HPV type in hybridization-positive biopsy specimens**						*HPV negative*	*Total*
	6	*11*	*Unclassified*	*31*	*16*	*18*		
Condyloma†	15	7	4	0	1‡	1	7	35
CIN 1	5	1	2	2	3	1	4	18
CIN 2	6	0	7	5	15	1	4	38
CIN 3	1	0	0	3	16	1	1	22
Cervical cancer	0	0	1	1	5	3	1	11
Total	27	8	14	11	40	7	17	124

$\chi^2 = 81.9$, $p = 0.001$. HPV = Human papillomavirus; CIN = cervical intraepithelial neoplasia.

*Unclassified and type 31 human papillomaviruses had a distribution pattern intermediate in severity to those of type 6 or 11 and type 16 or 18.

†Of the 35 "condylomas," 21 were clinically apparent exophytic lesions and 14 were flat subclinical lesions.

‡One condyloma contained type 16 plus type 18.

ysis. Each frozen sample was completely sectioned on a cryostat, such that multiple step sections were available as histologic controls, while the remaining slices were used for DNA analysis.

In the remaining 75 patients, less extensive sampling was performed, with biopsy of only the most severe areas of cervical or vulvar abnormality.

Histologic criteria. For study purposes, cervical or vulvar lesions were assigned within one of five grades, with the use of the following histologic criteria.

The term condyloma acuminatum was applied only to clinically visible exophytic papillomas showing a microscopic pattern of basal proliferation and koilocytosis within maturing surface layers. Macroscopically invisible lesions, recognizable colposcopically as an acetowhite reaction and showing the same histologic pattern as overt condylomas, were termed condyloma planum. Mild cytonuclear atypia, consistent with a diagnosis of very mild dysplasia, were often seen in both clinically apparent and subclinical lesions. The only difference between condyloma acuminatum and condyloma planum is that one is a macroscopically apparent papilloma while the other is an invisible epithelial proliferation recognizable only after soaking with acetic acid.[11] Preliminary statistical analysis indicated that both the papillary and the subclinical lesions showed the same type-specific viral associations. Hence, for subsequent analyses, both variants were grouped within a single category.

The diagnosis of cervical or vulvar intraepithelial neoplasia, grade 1, was reserved for epithelia in which the lower third was occupied by undifferentiated stem cells. Above the basal layer, surface differentiation was generally associated with koilocytotic atypia. A diagnosis of grade 1 intraepithelial neoplasia carried a connotation of sufficient cytonuclear atypia and architectural disorganization to justify categorization as a possible precursor. However, the distinction between condyloma versus grade 1 intraepithelial neoplasia was often difficult and subjective.

The category of cervical or vulvar intraepithelial neoplasia, grade 2, was reserved for architecturally disorganized epithelia, in which the basal two thirds was occupied by undifferentiated basal cells while those in the upper third displayed appreciable maturation. There was usually a clear distinction between grades 1 and 2, but considerable overlap sometimes existed between grades 2 and 3 intraepithelial neoplasias.

The diagnosis of grade 3 intraepithelial neoplasia was reserved for lesions displaying complete architectural disorganization, being composed of abnormal cells that showed minimal or absent surface differentiation. Invasive cancer was diagnosed according to standard criteria. Except for one case of microinvasion, all of these

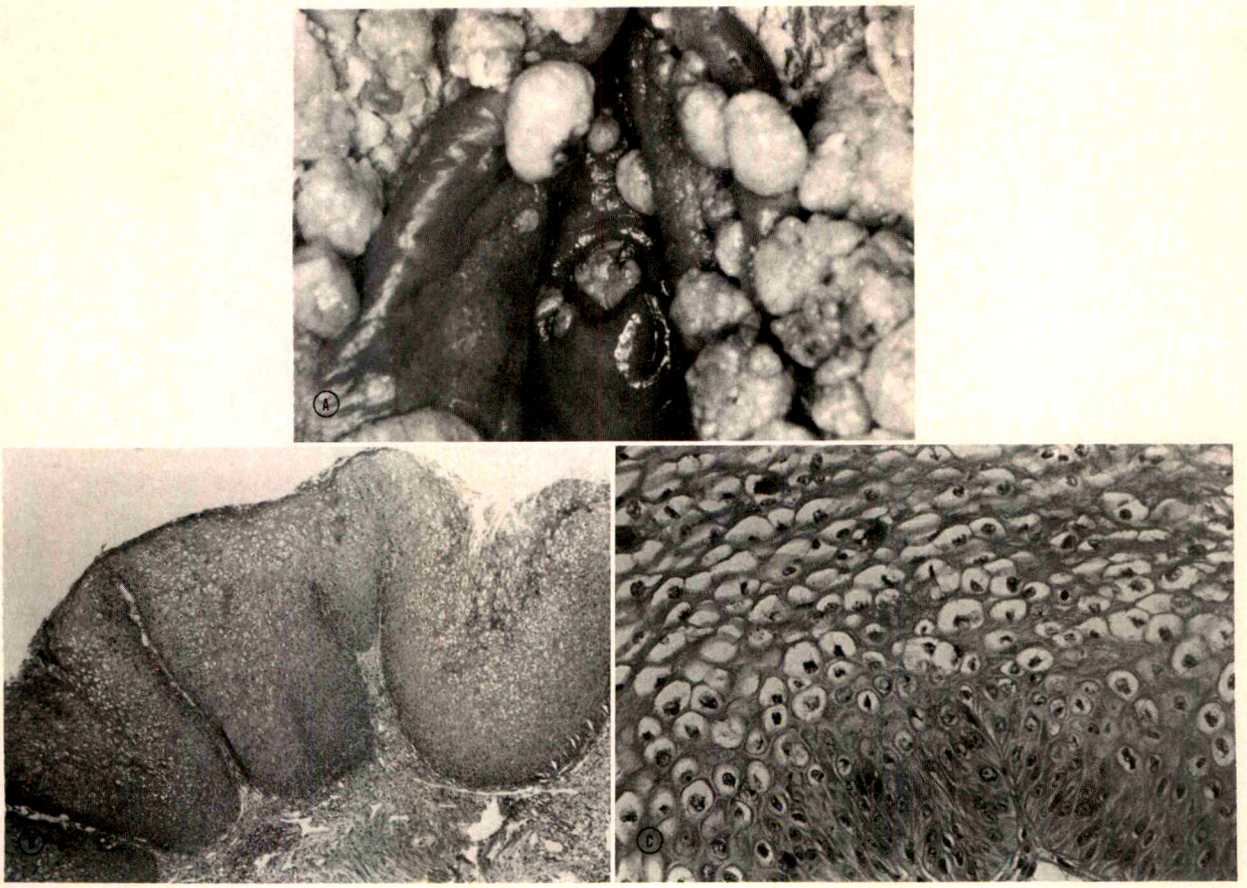


Fig. 1. Florid exophytic vulvar condylomas in an insulin-dependent diabetic patient, which were negative for papillomaviral DNA at hybridization. *A*: Colpophotograph. (Original magnification ×16.) *B*: Photomicrograph. (Hematoxylin and eosin. Original magnification ×13.) *C*: Photomicrograph. (Original magnification ×132.)

lesions were clinically overt malignancies treated by radical operations.

DNA hybridization. Samples were analyzed for viral DNA by Southern blot hybridization, as previously described.[10] For this procedure cellular DNA was extracted and reduced to small pieces by restriction enzyme digestion; the resulting fragments were separated according to size, by gel electrophoresis. The DNA fragments were denatured (meaning that double-stranded DNA was made single-stranded) and "blotted" onto a nitrocellulose filter. A panel of papillomaviral probes, each containing single-stranded, radiolabeled, type-specific viral DNA, was then incubated with the filter membrane under hybridization conditions, to allow annealing of the probes with any complementary segments of viral DNA present within the extracted cellular chromatin. The reaction was initially conducted under conditions of low stringency (meaning 35° C below the melting point of double-stranded DNA), to allow hybridization between any areas of partial nucleotide homology. After autoradiography, the filters were washed at high stringency (10° C below the duplex melting point of DNA) to destabilize any partial hybrids, such as those between the different papillomaviral types. The pattern of bands remaining on a second autoradiograph provided a sensitive and reliable guide to the type of any papillomaviral DNA present in the biopsy specimen. Results were then reconfirmed by high-stringency hybridization with probes derived from the individual papillomaviral types.

Data analysis. For each patient, the histologic grade and viral types of each of the multiple biopsy specimens were entered into a computerized data base and cross checked for accuracy. Data were analyzed on the Michigan Terminal System with the use of the Michigan Interactive Data Analysis System (MIDAS). This paper reports principally on the results of χ^2 testing of the associations between the major variables.

Results

Analysis of this data base revealed several relationships between viral type and either anatomic distribution or pathologic severity:

Papillomaviral DNA was detectable in the majority

Table III. Type-specific disease associations of the 94 principal vulvar biopsy specimens

Worst vulvar diagnosis	*HPV type in hybridization-positive biopsy specimens**						*HPV negative*	*Total*
	6	*11*	*Unclassified*	*31*	*16*	*18*		
Condyloma†	41	12	7	0	2‡	1	5	68
VIN 1	1	1	0	0	1	0	0	3
VIN 2	0	0	2	0	6§	0	0	8
VIN 3	0	0	0	0	9	0	1	10
Vulvar cancer	0	0	0	0	4	0	1	5
Total	42	13	9	0	22‡§	1	7	94

χ^2 = 115.3, p = 0.001. HPV = Human papillomavirus; VIN = vulvar intraepithelial neoplasia.

*Unclassified and type 31 human papillomavirus had a distribution pattern intermediate in severity to those of type 6 or 11 and type 16 or 18.

†Of the 68 "condylomas," 65 were clinically apparent exophytic lesions and three were flat, subclinical lesions.

‡One condyloma contained type 16 plus type 18.

§One grade 2 vulvar intraepithelial neoplasia lesion contained type 16 plus type 6 and another contained type 16 plus type 11.

Table IV. Type-specific anatomic patterns in 301 hybridization-positive biopsy specimens

Papillomaviral type	*Anatomic site*						*Total*
	Cervix		*Vagina*		*Vulva*		
	n	*%*	*n*	*%*	*n*	*%*	
6 or 11	39	29	19	53	87	67	145
Unclassified	20	15	5	14	13	10	38
31	14	10	1	3	0	0	15
16 or 18	62	46	11	31	30	23	103
Total	135	100	36	100	130	100	301

of lesions at low stringency. Low-stringency hybridization showed that 194 (89%) of the 218 principal cervical or vulvar biopsy specimens contained papillomaviral DNA of one type or another. Apart from a slightly lower detection rate (79%) in minor cervical lesions, positive hybridization results in the other lesions approximated 90% (Table I). Within each diagnostic category, no obvious histologic differences were observed between biopsy specimens giving either positive or negative hybridizations, suggesting that some apparently negative biopsy specimens may represent infection by unclassified viral types that do not react with the existing panel of probes (Fig. 1).

Type-specific disease patterns detectable at high stringency. Highly significant relationships were observed between "low-risk" types and minor lesions and between "high-risk" types and malignant lesions (χ^2 = 81.9 and 115.3, p = 0.000). On the cervix, type 6 or 11 was found in 22 of 35 (63%) condylomas and six of 18 (33%) grade 1 intraepithelial neoplasias (Table II). On the vulva, "low-risk" viruses were detected in 53 of 68 (78%) exophytic condylomas and two of three grade 1 intraepithelial neoplasias (Table III). In contrast, "high-risk" viruses were found mainly within high-grade intraepithelial and invasive lesions (Tables II and III). Type 16, 18, or 31 DNA was identified in 20 of 22 (91%) biopsy specimens of cervical intraepithelial neoplasia, grade 3, and nine of 11 (82%) invasive cervical cancers (including the adenosquamous carcinoma shown in Fig. 2). On the vulva, type 16 was found in 15 of 18 (83%) high-grade intraepithelial neoplasias and four of five cancers (80%). Nonetheless, it is important to note that 13 of 124 (10%) principal biopsy specimens from minor cervical or vulvar lesions contained "high-risk" papillomaviral types (Tables II and III).

Anatomic distribution of the different papillomaviral types. When 197 virus-containing principal biopsy specimens are combined with 104 secondary biopsy specimens that did not duplicate the viral types found in the principal samples, it is seen that most of the papillomaviral types can infect any of the anatomic sites (Table IV). However, type-specific trends were apparent. Types 6 and 11 were found in 67% of 130 vulvar biopsy specimens but only 29% of 135 cervical samples. In contrast, types 16 and 18 were detected in 46% of cervical specimens versus 23% of vulvar specimens. The unclassified types were almost equally distributed among the three sites. Type 31 was found in 10% of the cervical dysplasias but was not encountered on the vulva (Fig. 3).

Multicentric lesions may show differing viral pat-

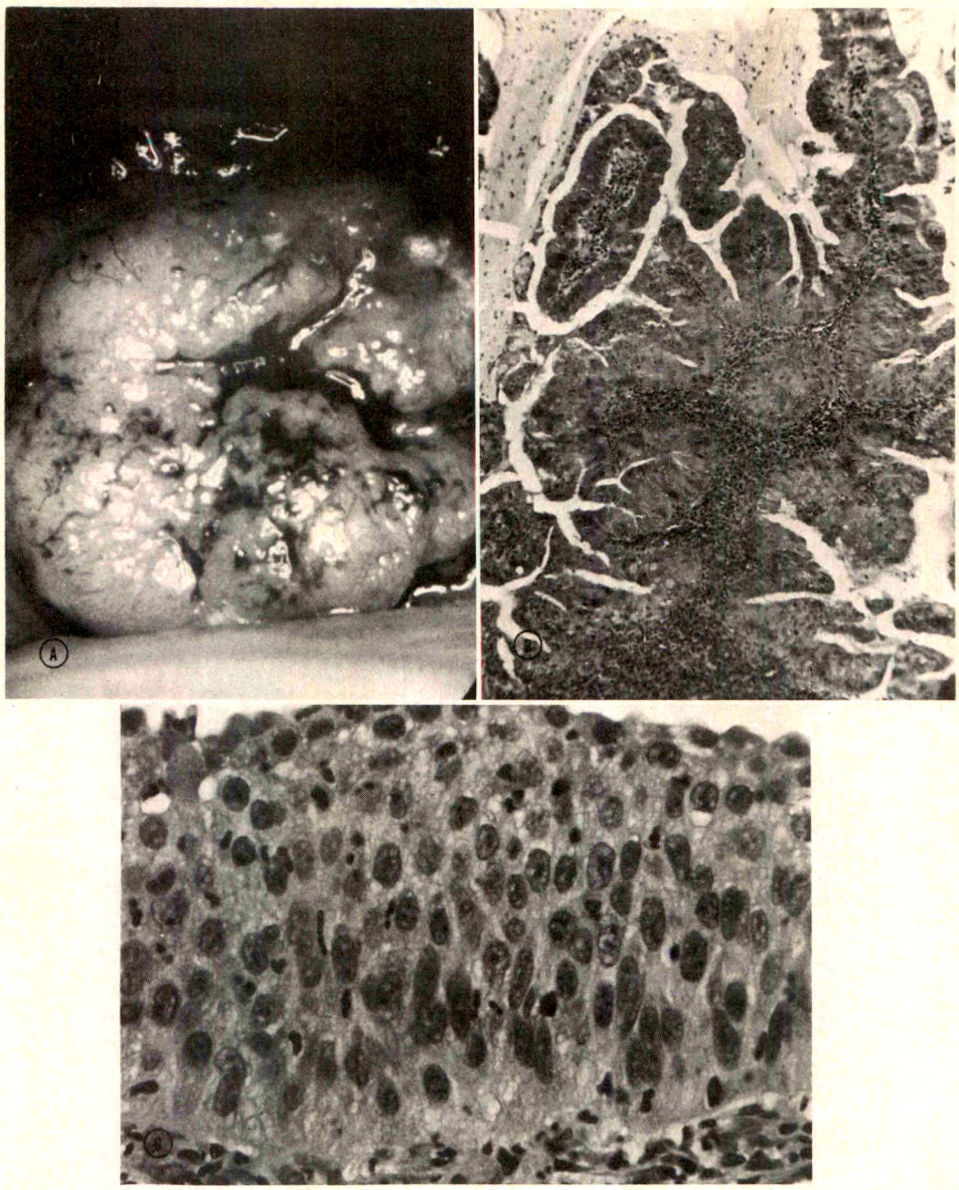


Fig. 2. A poorly differentiated adenosquamous carcinoma of the cervix that contained large amounts of papillomavirus type 18. *A*: Unmagnified photograph. *B*: Photomicrograph showing adenocarcinomatous element. (Hematoxylin and eosin. Original magnififcation ×33.) *C*: Photomicrograph of squamous element. (Hematoxylin and eosin. Original magnification ×132.)

terns. Among 58 women presenting with both cervical and vulvar disease, 46 had positive hybridization results at both sites. Thirty-two of these 46 samples (70%) had the same type of viral DNA in both samples and 14 (30%) showed different types (Table V). Multicentric lesions containing the same viral types tended to be of similar histologic grade. For example, 23 of 27 patients with minor lesions had the same papillomavirus within both samples, as did five of six patients with grade 2 or 3 intraepithelial neoplasia affecting both cervix and vulva. In contrast, only 43% of lesions containing different viruses were of similar histologic grade, with two, three, and four levels of disparity being found in 21%, 29%, and 7%, respectively (Fig. 4).

Infrequent occurrence of mixed papillomaviral infections. Although different papillomaviruses were frequently encountered at different sites within the same patient, it was uncommon to find more than one viral type within a single biopsy specimen. Multiple viral types were found at different sites in 30 women (14 principal biopsy specimens from women with multicentric lesions and 16 secondary biopsy specimens taken from women sampled according to the original protocol). In contrast, only six (2%) of 301 hybridiza-

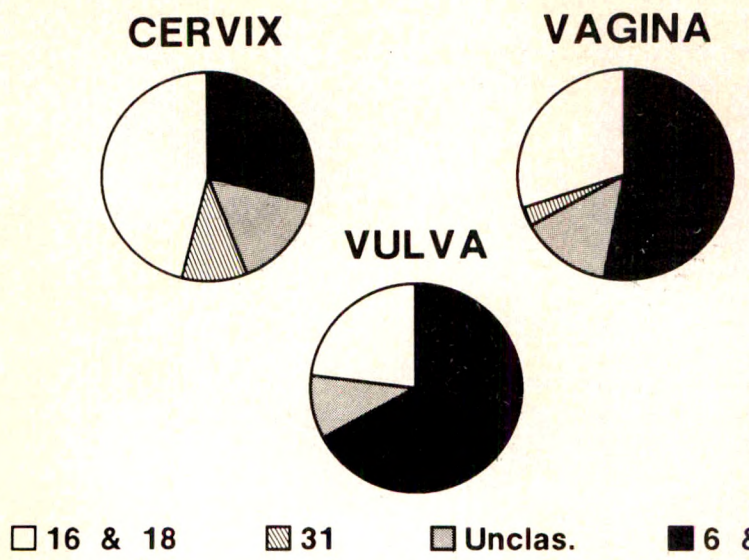


Fig. 3. A pie chart depicting the anatomic distribution of the different papillomaviral types.

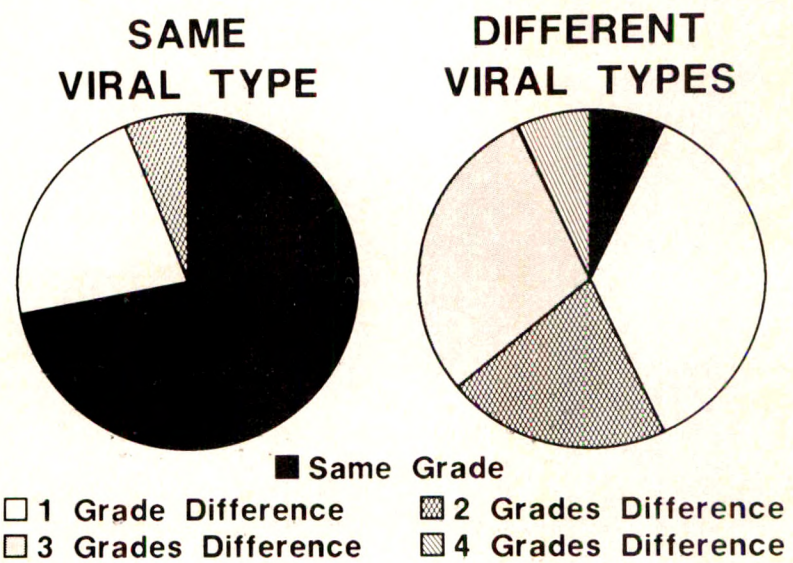


Fig. 4. A pie chart comparing histologic grades of cervical and vulvar lesions, according to whether both biopsy specimens contained the same type or different types of papillomaviral DNA.

tion-positive samples displayed more than one papillomaviral type within an area of uniform colposcopic appearance. Three of these six mixed infections, showing a combination of types 16 and 18, were obtained from histologically similar condylomas affecting the cervix, vagina, and vulva of the same patient (Fig. 5). A fourth example of mixed infection by types 16 and 18 was found within an area of minimally acanthotic vaginal epithelium, collected from a patient with type 6 associated cervical and vulvar condylomas. The two remaining examples were vulvar condylomas that contained a combination of type 16 plus type 6 or 11. Although these exophytic lesions were unremarkable from the clinical viewpoint, biopsy specimens from both patients showed foci of grade 2 intraepithelial neoplasia.

Latent papillomaviral infection in adjacent tissue. Latent papillomaviral infection was found in a substantial proportion of the histologically normal (or only minimally abnormal) secondary biopsy specimens. Detection rates at the various sites were as follows: 36 of 52 (69%) specimens from minor grade acetowhitening on the vulva; 16 of 25 (64%) areas of minor grade acetowhitening adjacent to more significant lesions

Table V. How multicentric lesions arise

Presenting lesion	*Second site*			*Total*
	Same HPV	*Different HPV*	*Negative*	
HPV 6 and 11	22	2	4	28
Unclassified HPV	4	0	4	8
HPV 16 and 18	6	12	0	18
First-site negative	0	0	4	4
Total	32	14	12	58

HPV = Human papillomavirus.

within the transformation zone; 34 of 80 (42%) specimens of vaginal epithelium showing a colposcopic pattern characterized by multiple, small acetowhite spikes ("asperities") projecting above an otherwise pink vaginal mucosa; two of eight (25%) specimens of normal-appearing immature squamous metaplasia, collected proximal to the new squamocolumnar junction in patients with cervical intraepithelial neoplasia containing type 16 or 18; and five of 33 (15%) specimens from normal-appearing skin at the intended margin of laser destruction (Table VI). Within each category, no histologic differences were observed between virus-positive and virus-negative samples.

The five patients who had viral DNA detectable in biopsy specimens of colposcopically normal vulvar skin had the most extensive disease, as reflected by the fact that all of the 18 additional principal or secondary biopsy specimens from these five women gave positive hybridization results. Two of these patients had immune deficiencies and two others had severe diabetes. Conversely, among the group in whom the samples of normal-appearing vulvar skin gave negative hybridization results, papillomaviral DNA was detectable in only 57 of the other 102 (56%) secondary biopsies collected from these women ($\chi^2 = 10.9$, $p = 0.001$).

Comment

Results of this study confirm that papillomaviral DNA (of one type or another) can be detected in most neoplastic lesions of the lower genital tract, irrespective of anatomic site or pathologic grade. Specific papillomaviruses were associated with specific disease patterns, suggesting that this relationship is causal rather than coincidental. For the purposes of examining broad differences in the biologic significance of the specific viral types, it is useful to combine both cervical and vulvar lesions into a single table. Grouping the principal cervical and vulvar biopsy specimens as benign to minimally atypical, partial thickness intraepithelial neoplasia, or fully neoplastic (with or without invasion) revealed that only one of 90 lesions (1%) containing type 6 or 11 had a diagnosis greater than grade 2 intraepithelial neoplasia (Table VII). Conversely, 38 of 70 samples (54%) containing type 16 or 18 DNA displayed an unequivocally malignant phenotype (Fig. 6). Type 31 and the unclassified group showed an intermediate pattern, being detectable in lesions that, on javerage, were significantly worse than those seen with types 6 and 11 but significantly less severe than those seen with types 16 and 18 ($\chi^2 = 1120.3$, $p = 0.001$).

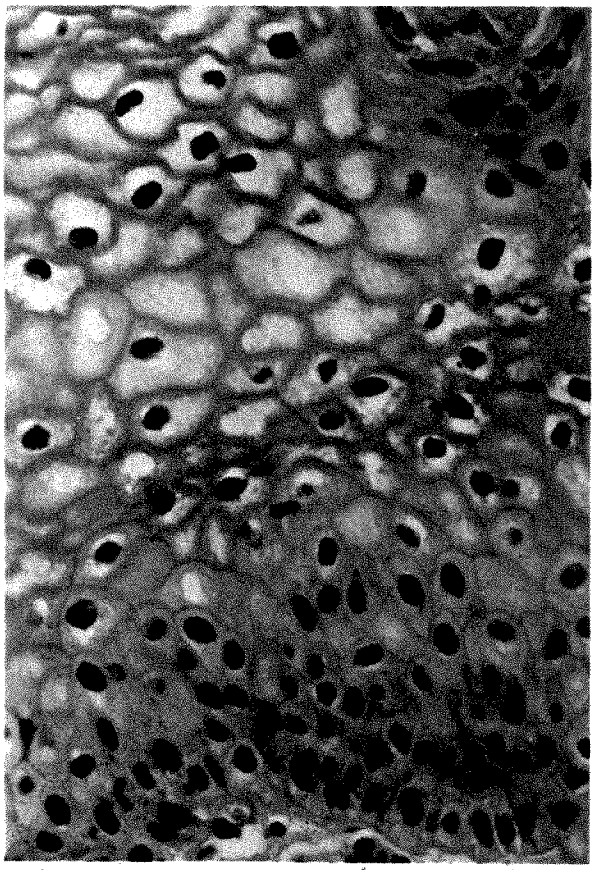


Fig. 5. A bland-appearing condyloma containing papillomaviral types 16 and 18. (Hematoxylin and eosin. Original magnification ×132.)

Events in the individual patient remain a mystery. Why the clinical consequences of papillomaviral infection should be generalized in some patients, yet localized to differing anatomic sites in others, is a major puzzle. The distribution patterns of the papillomaviruses detected in this study showed that the majority of types were detectable throughout the lower genital tract (Fig. 3). Nonetheless, type-specific differences in both site and severity were discernible. First, anatomic patterns showed some relationship to viral type. Whereas type 16 was detected in 36 of 71 high-grade cervical lesions and 20 of 26 vulvar neoplasias, type 31 was not found in any of 180 vulvar biopsy specimens and type 18 was not encountered among 26 vulvar

Table VI. Stepwise correlation between degree of pathologic expression and detectability of papillomaviral DNA

Biopsy category	*Biopsy site*	*Colposcopic appearance*	*Histologic features*	*HPV DNA detection rate (%)*
Principal, vulvar	Worst area of vulvar abnormality	Definite condylomatous or neoplastic lesion	Fully expressed koilocytotic atypia with or without neoplastic features	93
Principal, cervical	Worst area of cervical abnormality	Definite condylomatous or neoplastic lesion	Fully expressed koilocytotic atypia with or without neoplastic features	86
Secondary, vulvar lesion	Minor-grade acetowhitening of medial labia minora	Questionable lesion	Acanthosis, capillary proliferation, occasional vacuolated cells	69
Secondary, cervical lesion	Minor acetowhitening of cervical transformation zone	Questionable lesion	Acanthosis, capillary proliferation, occasional vacuolated cells	64
Secondary, vaginal walls	Vaginal epithelium, sampling of "asperities" if present	Questionable lesion	Acanthosis, capillary proliferation, occasional vacuolated cells	42
Secondary, normal metaplasia	Proximal to new squamocolumnar junction	Normal immature metaplasia	Normal immature metaplasia	25
Secondary, normal skin	Laser vulvectomy margin	Normal skin of labia majora	Normal skin	15

Table VII. Type-specific viral associations within the 218 principal biopsy specimens from cervix and vulva

Papillomaviral type	*Condylomas, CIN 1, and VIN 1*	*CIN 2 and VIN 2*	*CIN 3, VIN 3, and invasive cancer*	*Total*
No virus found	16	4	4	24
HPV 6 and 11	83	6	1	90
Unclassified HPV	13	9	1	23
HPV 31	2	5	4	11
HPV 16 and 18	10	22	38	70
Total	124	46	48	218

$\chi^2 = 120.3$, $p = 0.001$. CIN = Cervical intraepithelial neoplasia. VIN = Vulvar intraepithelial neoplasia. HPV = Human papillomavirus.

neoplasias. Second, the most florid manifestations of "low-risk" infections were seen on the introital mucosae or the labial and perineal skin, whereas the most severe effects of "high-risk" infections generally occurred on the cervix. For example, of 35 patients with type 6 or 11 lesions of the transformation zone, only 31% presented because of an abnormal Papanicolaou smear. The remainder were discovered during colposcopic examination of women seen for vulvar condylomas. Conversely, 93% of 58 women with type 16, 18, or 31 infections of the transformation zone presented with either abnormal cervical cytologic test results or symptomatic cancers. Hence individual variation in anatomic distribution and disease severity may be due, in part, to site-specific predilections of the different viral types.

Individual variations in anatomic extent and clinical course also appear to reflect profound differences in the effectiveness of host response. The detection of latent papillomaviral infections in 55% of secondary biopsy specimens collected from adjacent areas of minor grade acetowhitening and within 15% of colposcopically normal samples from the intended treatment margin indicates that viral genetic information is a necessary but not sufficient cause for condyloma formation or neoplastic change (Table VI). At least for the subset of patients in whom a single type of papillomaviral DNA was detectable at multiple sites, cell-virus interaction seems to be regulated by local factors. In short, does localized disease represent focal breakdown of host control within a field of diffuse papillomaviral infection?

Another puzzling feature of papillomaviral infection in the individual patient is the question of how multicentric disease arises. Of the 58 women with concurrent cervical and vulvar lesions, 32 arose through diffuse infection by a single viral type and 14 had multiple infections by two, three, or four papillomaviruses (Table V). Lesions of similar histologic grade generally contained the same virus, while those displaying marked disparity tended to show different types (Fig. 4). Nonetheless, reliable determination was not possible on clinical grounds.

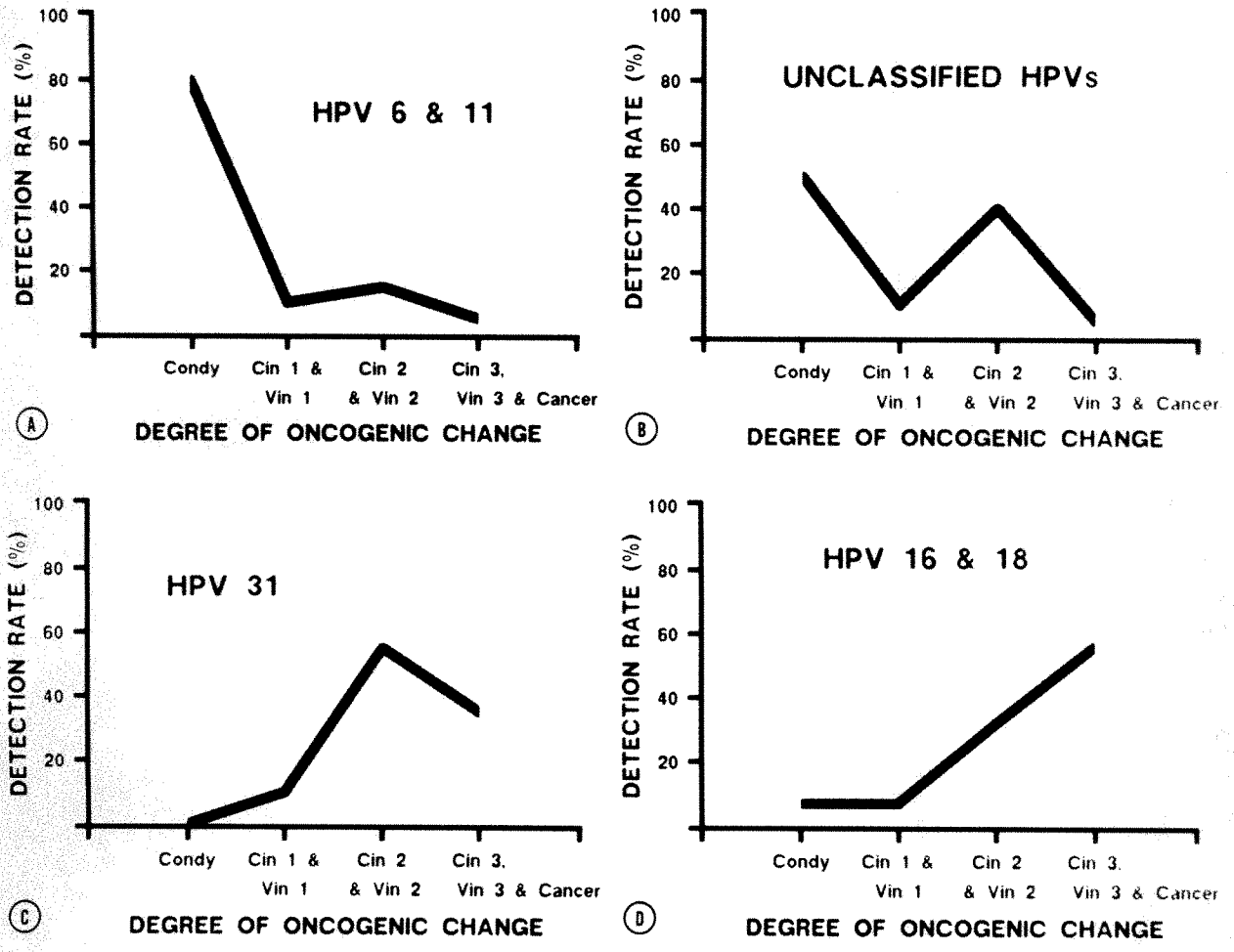


Fig. 6. A bar chart comparing the differing severities of histologic change associated with the different papillomaviral types. *A*: Human papillomavirus type 6 or 11. *B*: Unclassified types. *C*: Human papillomavirus type 31. *D*: Human papillomavirus type 16 or 18.

The fundamental reason for collecting multiple biopsy specimens from adjacent tissues was to test the hypothesis that preexisting type 6 or 11 infection might predispose to the subsequent entry of type 16, 18, or 31, thereby implicating the so-called "low-risk" types as facilitory factors. We found no evidence of any such relationship. Rather, when multiple papillomaviruses were isolated from a single patient, different types were generally confined to sharply demarcated areas of differing histologic appearance. In short, prior infection by one papillomavirus may actually resist subsequent superinfection by a second type. The only discernible relationship between the different groups appears to be that these various viruses display a morphologic resemblance and share a common mode of transmission.

Whether histologic examination can differentiate "high-risk" from "low-risk" disorders remains controversial. Some workers report that type 16–associated lesions are recognizable by the presence of atypical mitotic figures.[12] Others have emphasized that papillomaviral infection produces a spectrum of morphologic change, extending from condyloma to cancer.[13] Within this study, only one lesion having a diagnosis of severe dysplasia or worse was found to contain type 6 or 11 DNA. However, the converse was not true. Overemphasizing the association between the "high-risk" group and atypical mitotic figures would have led us to overlook the 10% of minor lesions associated with type 16, 18, or 31, including several bland-appearing overt condylomas (Fig. 5). Morphologic overlap was greatest in middle-grade lesions, as reflected by the detection of "high-risk" types in 59% and "low-risk" or unclassified types in 32% of grade 2 intraepithelial neoplasias (Table VII).

Treatment of vulvar condylomas should take account of the phases in the natural history of papillomaviral infection. Contact tracing studies suggested that, during an incubation period of 6 weeks to 8 months, latent viral infection becomes widely established within the lower genital tract.[14] Thereafter, rapid epithelial and capillary

proliferation often results in focal papilloma formation. For patients seen early in the active phase, the clinician should understand that much of the apparent "spread" of disease really represents differing rates of evolution from latent to active infection at different sites. After about 3 months, lesion growth generally slows, presumably as a result of the host immune response.[2] Although essentially all condylomas are surrounded by diffuse areas of subclinical infection, destruction of just the exophytic lesions will lead to stable remission in about 85% of women. Hence laser therapy should be reserved for women in whom lesions are too extensive for control by caustic agents and for those who do not respond to simpler therapies. Once the decision to use the laser has been made, it is difficult to see what is achieved by "spot welding" only the exophytic condylomas. Prior clinical studies[15] indicate that to avoid excessive rates of lesion recurrence, the approach should be to perform extended laser destruction, encompassing all areas of colposcopically suspected papillomaviral infection (as indicated by acetowhitening).

Opinion is divided as to the best strategy for managing cervical condylomas. Given the prevalence of "high-risk" types in condyloma planum, the susceptibility of metaplastic epithelium to malignant transformation, and the innocuous nature of outpatient therapies, there is much to commend a policy of treating all cervical lesions by transformation zone ablation.[16] While it is conceded that the majority of minor cervical lesions are not genuine precursors, effective treatment of all permissive infections would appear to be a reasonable step toward the control of western society's burgeoning epidemic of sexually transmitted papillomaviral infections.

In conclusion, a definite association was confirmed between papillomaviral type and pathologic grade. Types 6 and 11 were seldom found in bona fide neoplasias, whereas types 16, 18, and 31 were most commonly seen in cancers and definite premalignancies. Nonetheless, some 10% of clinically unremarkable lesions contained types 16, 18, and 31. There appeared to be a stepwise correlation between the degree of histologic disturbance and the frequency of positive hybridizations, papillomaviral DNA being detectable in 89% of the principal cervical and vulvar biopsy specimens (showing florid pathologic features), 55% of the minimally acetowhite secondary biopsy specimens (showing mild acanthosis, capillary proliferation, and occasional vacuolated cells) and 15% of the colposcopically normal secondary biopsy specimens (showing unremarkable vulvar skin or immature squamous metaplasia). It is postulated that individual variations in disease distribution and clinical course are explained partly by site-specific predilections of the particular viral types and partly by differences in host response. Given the chronicity of papillomaviral infections and the ease with which latent viral DNA can be detected in adjacent tissues, the major mechanism of treatment failure may be reexposure of the healing area to a viral reservoir in "normal" epithelium. Whether untreated male partners also contribute to this viral reservoir remains an open question.

We gratefully acknowledge Allison Quinn, B.S., Lai Lim, B.A., and Guy Campbell, M.S., for technical assistance, Harald zur Hausen, M.D., for making available several viral probes, and Robert Kurman, M.D., and Wayne Lancaster, Ph.D., for helpful consultation.

REFERENCES

1. Pfister H. Biology and biochemistry of papillomaviruses. Rev Physiol Biochem Pharmacol 1983;99:111.
2. Reid R, Stanhope CR, Herschman BR, Booth E, Phibbs G, Smith JP. Genital warts and cervical cancer. I. Evidence of an association between subclinical papillomavirus infection and cervical malignancy. Cancer 1982; 50:377.
3. Gissmann L. Papillomaviruses and their association with cancer in animals and in man. Cancer Surv 1984;3:161.
4. Lancaster WD, Olson C. Animal papillomavirus. Microbiol Rev 1982;46:191.
5. Orth G, Breitburd F, Favre M, Croissant O. Papillomaviruses: possible role in human cancer. In: Watson J, ed. Origins of human cancer. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, 1977:1043.
6. Gissmann L, Wolnik L, Ikenberg H, Koldovsky U, Schnürch HG, zur Hausen H. Human papillomavirus types 6 and 11 DNA sequences in genital and laryngeal papillomas and in some cervical cancers. Proc Natl Acad Sci USA 1983;80:560.
7. Durst M, Gissmann L, Ikenberg H, et al. A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. Proc Natl Acad Sci USA 1983;80:3812.
8. Boshart M, Gissmann L, Ikenberg H, et al. A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. EMBO 1984;3:1151.
9. Ikenberg H, Gissmann L, Gross G, et al. Human papillomavirus type 16-related DNA in genital Bowen's disease and in Bowenoid papulosis. Int J Cancer 1983;32:563.
10. Lorincz AT, Lancaster WD, Temple G. Cloning and characterization of the DNA of a new human papillomavirus from a woman with dysplasia of the uterine cervix. J Virol 1986;58:225.
11. Reid R, Scalzi P. Genital warts and cervical cancer. VII. An improved colposcopic index for differentiating benign papillomaviral infections from high-grade cervical intraepithelial neoplasia. Am J Obstet Gynecol 1985;153:611.
12. Crum CP, Ikenberg H, Richart RM, et al. Human papillomavirus type 16 and early cervical neoplasia. N Engl J Med 1984;310:880.
13. Reid R, Fu YS. Is there a morphologic spectrum linking condyloma to cervical cancer? In: Banbury report no.21: viral etiology of cervical cancer. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, 1986:91.
14. Oriel JD. Natural history of genital warts. Br J Vener Dis 1971;47:1.
15. Reid R, Elfont EA, Zirkin RM. Superficial laser vulvectomy. II. The anatomic and biophysical principles permitting accurate control of the depth of dermal destruction with the carbon dioxide laser. Am J Obstet Gynecol 1985;151:261.
16. Reid R. Symposium on cervical neoplasia. V. Carbon dioxide laser ablation. Colposc Gynecol Laser Surg 1985; 1:291.

Yolk sac tumors of the ovary and the human yolk sac

Toshimitsu Takashina, M.D., Yuugi Kanda, M.D., Osamu Hayakawa, M.D., Ryuichi Kudo, M.D., Eiki Ito, M.D., and Satoru Sagae, M.D.
Sapporo, Hokkaido, Japan

In the present study a comparison was made between human yolk sacs and yolk sac tumors. Tubules surrounded by several to as many as 10 endodermal cells and intracellular tubules in one endodermal cell were frequently observed. The tubules were seen abundantly in the yolk sac of a 4-week pregnancy, and they resembled the reticular pattern of the yolk sac tumor. It was also observed that the papillary endoderm, which contained blood cells in the center and protruded into the endodermal tubules, resembled the Schiller-Duval body of yolk sac tumor. Ultrastructurally the tumor cells were similar to the yolk sac endoderm. α-Fetoprotein was positive in the yolk sac endoderm until approximately the seventh week of pregnancy. Yolk sac tumor was also α-fetoprotein–positive. In other words, our study of human yolk sacs of 4- to 11-week pregnancies presumes that the yolk sac tumor resembles the endoderm of 4- to 7-week pregnancies. (Am J Obstet Gynecol 1987;156:223-9.)

Key words: Yolk sac tumor, endodermal sinus tumor, human yolk sac

Schiller[1] reported on a tumor group with a specific histologic picture showing a glomerulus-like structure and termed the tumor "mesonephroma ovarii." Teilum[2] termed the tumor "endodermal sinus tumor" from the fact that the glomerulus-like structure resembles the endodermal sinus in the placenta of rats observed by Duval.[3] The theory of the histogenesis of the germ cell tumor proposed by Teilum has been supported, and the tumor has been termed "yolk sacs tumor" by Teilum et al.,[4, 5] and Huntington and Bullock.[6, 7]

A morphologic similarity of the yolk sac tumor to the human yolk sac has been pointed out.[8, 9] In the present study a morphologic comparison was made between the tumor and the human yolk sac in various stages of pregnancy, and in addition, a functional comparison of the producibility of α-fetoprotein was performed to study the histogenesis of the tumor.

Material and methods

We have seen 14 cases of yolk sac tumor in our department during the past 24 years. Light microscopic investigations were carried out on these 14 cases, seven of which were electron microscopically observed. After informed consent was received, the yolk sacs were obtained from pregnant patients who had undergone hysterectomies because of uterine myoma or cervical cancer and from those who had undergone elective abortion during the past 8 years. Yolk sacs were obtained from a total of 27 cases: one at pregnancy week 4, three at week 5, four at week 6, nine at week 7, five at week 8, two at week 9, two at week 10, and one at week 11.

For light microscopy the extirpated tissue fragments were fixed in 95% ethanol or 10% buffered formalin. Paraffin sections were treated with hematoxylin-eosin, Giemsa, or periodic acid–Schiff (PAS) staining. For electron microscopy, small pieces of tissue were fixed in 2.5% glutaraldehyde in 0.1 mol/L of cacodylate buffer, pH 7.4, for 1 hour and were postfixed in 0.2% osmium tetroxide in the same buffer for 1 hour. The samples were embedded in Epon 812 after dehydration in a graded ethanol series. Ultrathin sections were prepared with the aid of LKB 2088 Ultratome V and stained with uranyl acetate and lead citrate. Semithin sections adjacent to the electron microscopic sections were also prepared from the Epon-embedded material and stained with toluidine blue for light microscopic examinations. To investigate α-fetoprotein, the tissue fragments of the extirpated yolk sac tumors and the human yolk sacs were fixed in 95% alcohol. Paraffin sections were prepared and immunohistochemical localization of α-fetoprotein was carried out with use of the peroxidase antiperoxidase method.[10, 11] In addition, to confirm the specificity of α-fetoprotein, the cervix uteri, endometrium, and dysgerminoma were used as controls.

From the Department of Obstetrics and Gynecology, Sapporo Medical College.
Received for publication May 26, 1986; accepted August 19, 1986.
Reprint requests: Toshimitsu Takashina, M.D., Department of Obstetrics and Gynecology, Sapporo Medical College, South 1, West 16, Chuo-ku, Sapporo 060, Hokkaido, Japan.

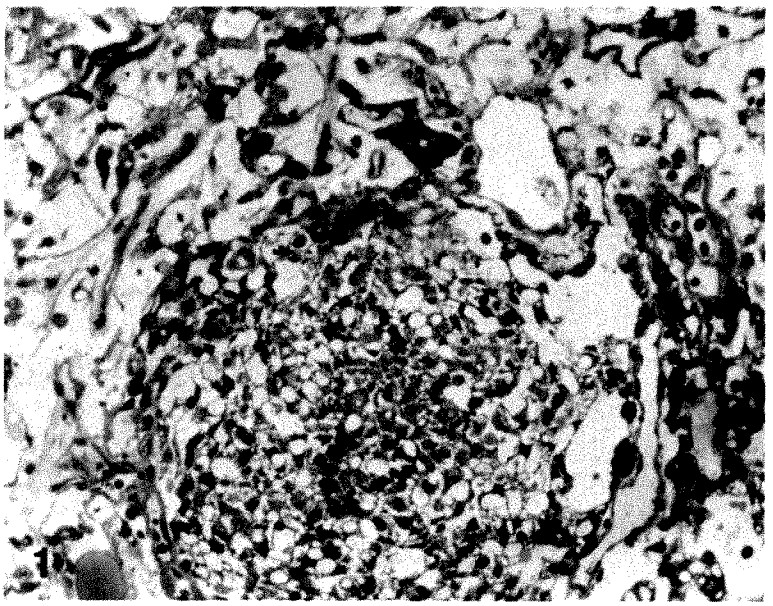


Fig. 1. Cystic and microcystic structures fused mutually and formed a reticular pattern (toluidine blue staining).

Results

Ovarian yolk sac tumor

Light microscopic findings. The highly atypical tumor cells showed various histologic structures that were characterized by a radiated arrangement of tumor cells centering around blood vessels. The structure resembled a glomerulus-like structure, and this was designated as an endodermal sinus pattern or a Schiller-Duval body. Around this structure, cystic and microcystic structures frequently were fused together, and a picture resembling a reticular pattern was seen (Fig. 1). Furthermore, undifferentiated small cells were closely arranged to form a solid pattern. Hyaline globules were seen intracellularly and extracellularly and were PAS stain–positive in resistance to diastase digestion. Amorphous substances having a similar stainable property were seen extracellularly. However, only a few parts of the polyvesicular vitelline pattern were observed. The other tumor components were complicated by dysgerminoma, cystic teratoma, and Brenner tumor in two, four, and one patients, respectively.

Electron microscopic findings. Cytoplasmic protrusions of tumor cells were seen between the vascular endothelial cells of the Schiller-Duval body and the tumor cell, and a basement-membranelike material having moderate electron density was seen around the protrusions. The adjacent tumor cells were bound by a tight junction and desmosomes. Short microvilli were observed on the cell surface and in the magnified intercellular cavity. The nucleus was elliptic or irregular in shape and had one to two nucleoli exhibiting nucleolonema. The cytoplasm was abundant in organelles, and many free ribosomes and glycogen granules were distributed all over the cytoplasm along with glycogen conglomerates at the border of the cytoplasm. Many ovoid mitochondria, closely related to the rough endoplasmic reticulum, were also noted.

Tumor cells forming structures other than that of the Schiller-Duval body consisted of similar cellular organelles and closely resembled the tumor cells forming the Schiller-Duval body; that is, the tumor cells were sparsely arranged, had cytoplasmic protrusions, and were bound by a tight junction and desmosomes. Microvilli were seen in the enlarged intercellular cavity and in the tubule in one tumor cell. The nucleus was large and irregular in shape and had well-defined nucleoli. Cellular organelles were abundant, and rough endoplasmic reticulum was arranged in a parallel manner and often surrounded by ovoid mitochondria, moreover, abundant rosettelike accumulations of glycogen granules were observed (Fig. 2). Occasionally a well-developed Golgi apparatus was seen, and in addition, ovoid amorphous substances having high electron density were observed near the rough endoplasmic reticulum. Extracellularly an amorphous substances surrounding the tumor cells was observed. This substance was the same as the basement-membranelike material seen in the Schiller-Duval body.

As mentioned previously, in light microscopic studies the tumor represented varying histologic pictures, but essentially it consisted of tumor cells having the same fundamental structure when viewed with an electron microscope.

Human yolk sac. A comparison of yolk sac tumor

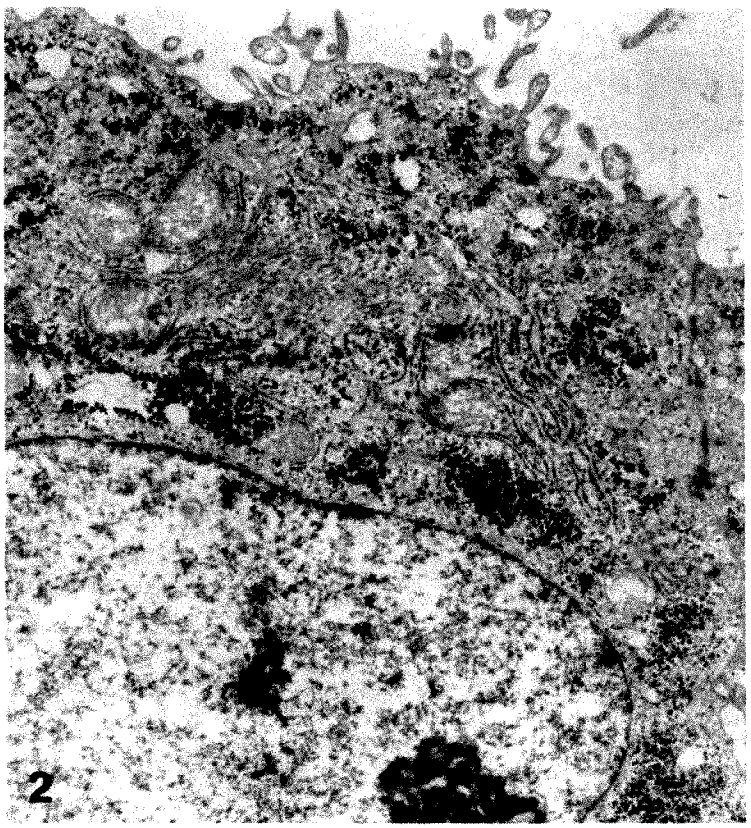


Fig. 2. A reticular pattern of tumor cells. Microvilli were seen on the cell surface. Desmosomes were seen. The nucleus had a nucleolus exhibiting nucleolonema. Ovoid mitochondria were closely in contact with parallelly arranged rough endoplasmic reticulum. Glycogen fields were observed as being dispersed. (× 10000.)

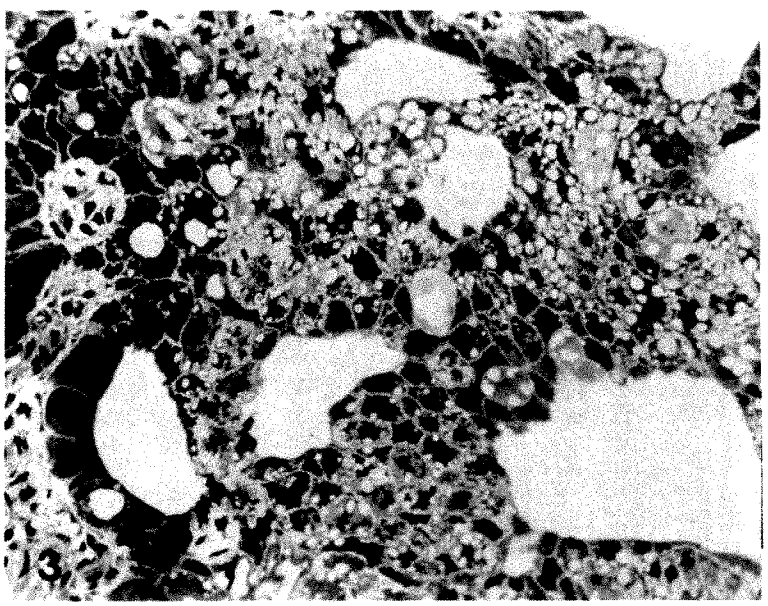


Fig. 3. Many endodermal tubules and intracellular tubules were seen in the endoderm layer (fourth week of pregnancy; toluidine blue staining).



Fig. 4. Blood cells with a large nucleus were observed in the center of the endoderm protruding into the endodermal tubule (sixth week of pregnancy; toluidine blue staining).

findings, mainly those of the yolk sac endoderm, was made.

Light microscopic findings. The human yolk sac wall consisted of three layers: an endoderm facing the yolk sac cavity, a mesothelium facing the chorionic cavity, and a mesenchyme situated between them. The endodermal cells were cuboidal and displayed a flagstone-like arrangement. Several to approximately 10 endodermal cells were arranged to form tubular structures (endodermal tubules), and a formation of microtubular structures also occurred within the cytoplasms of the endodermal cells (intracellular tubules). In the yolk sac of a 4-week pregnancy, many endodermal tubules and intracellular tubules were seen in the endodermal layer (Fig. 3). A structure in which the papillary endoderm protruded into the endodermal tubules was seen in some yolk sacs of 6- and 7-week pregnancies. Large blood cells were occasionally noted in the center of the papillary endoderm. This structure was found to be spherical when observed in the serial sections (Fig. 4). PAS staining after diastase digestion was positive until approximately the seventh week of pregnancy but negative thereafter.

Electron microscopic findings. Short microvilli were regularly arranged on the cell surface. Intercellularly, many slender cytoplasms protruded microvillously. The cells were bound by desmosomes and a tight junction. The nucleus was generally ovoid and possessed nucleoli exhibiting nucleolonema. The cytoplasm was abundant in organelles, and the rough endoplasmic reticulum showed several 10-layered parallel arrangements. Glycogen granules were abundant. The formation of a glycogen field and a rosette by glycogen granules were observed. Ovoid mitochondria were present in close contact with the rough endoplasmic reticulum. Free ribosomes and Golgi apparatus were also observed as being dispersed. On the cell surface facing the endodermal tubules or intracellular tubules, microvilli were seen (Fig. 5). A basement membrane was noted between the endoderm and mesenchyme in some yolk sacs of 6- and 7-week pregnancies (Fig. 6). A spherical amorphous substance with a high electron density was observed in the cytoplasm of endodermal cells until the fifth to ninth week of pregnancy (Fig. 5).

α-Fetoprotein findings. Of 14 patients with yolk sac tumor, preoperative α-fetoprotein values in six patients (whose serum α-fetoprotein was measured by radioimmunoassay) were all found to be positive. α-Fetoprotein values increased or decreased before and after surgery and at the time of chemotherapy as well as upon recurrence. A study of the immunohistochemical localization of α-fetoprotein produced negative results in the cervix uteri, endometrium, and dysgerminoma, which were selected as controls; α-fetoprotein, however, was positive in the tumor cells of the yolk sac tumors. In the human yolk sac, α-fetoprotein was positive in all the layers of the yolk sac endoderm until the seventh week of pregnancy (Fig. 7). There were α-fetoprotein–positive cases in some yolk sacs after 8 weeks of pregnancy. Neither the mesothelium nor the mesenchyme gave positive findings. These modes of appearance of α-fetoprotein were nearly coincident

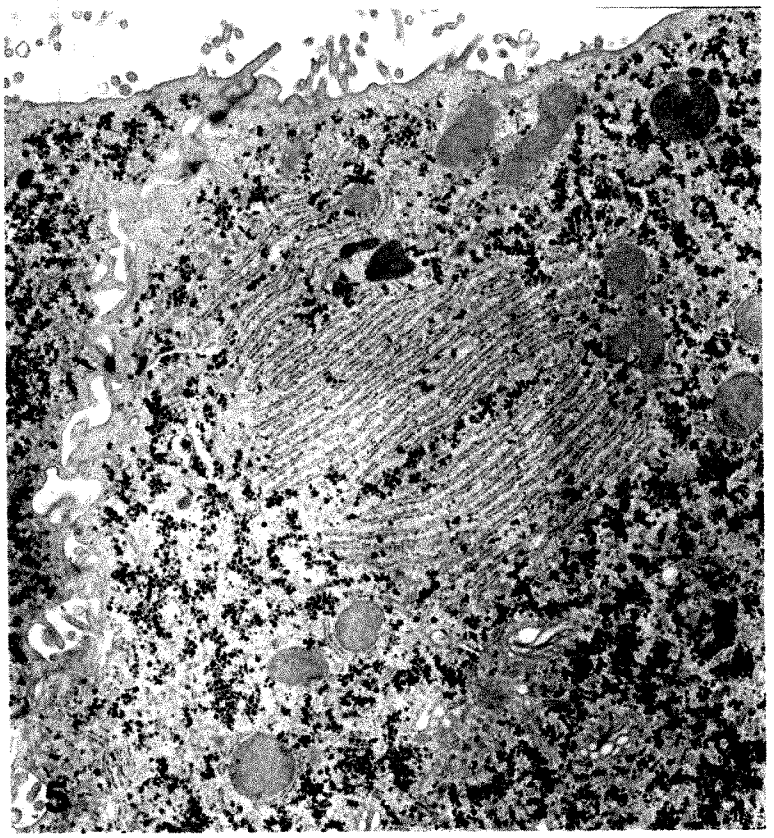


Fig. 5. Microvilli were seen on the cytoplasmic surface. Parallelly arranged rough endoplasmic reticulum was adjacent to mitochondria. Glycogen was abundant and distributed all over the cytoplasma. A spherical amorphous substance was seen near the rough endoplasmic reticulum (sixth week of pregnancy). (×5000.)



Fig. 6. Basement membrane was observed at the border between the superior endoderm and the inferior mesenchyme *(arrows)* (sixth week of pregnancy). (×16000.)

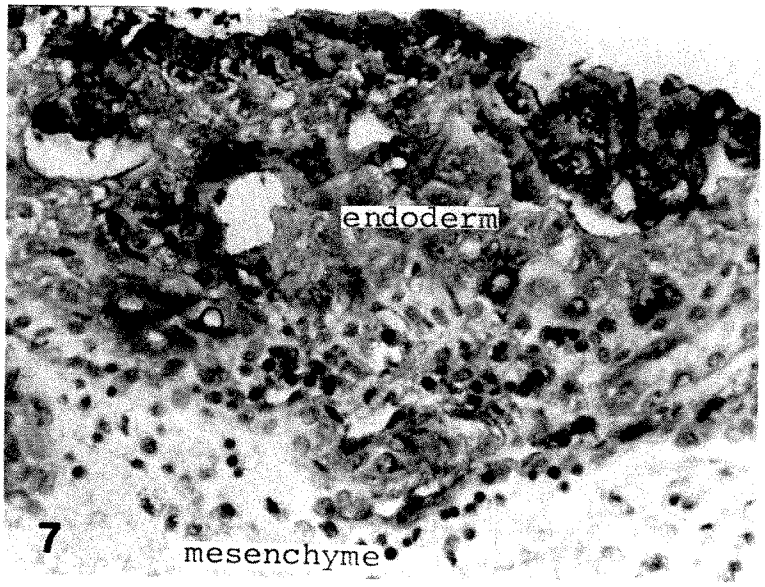


Fig. 7. α-Fetoprotein was observed as a brownish yellow granule in all the layers of the yolk sac *endoderm* of a 6-week pregnancy. *Mesenchyme* was α-fetoprotein–negative.

with those of the substance that was PAS stain–positive after diastase digestion.

Comment

With respect to the histogenesis of the yolk sac tumor, Teilum et al.[2, 4, 5] compared the histologic picture with viviparous tissue and proposed a histogenesis theory of the germ cell tumor. Huntington and Bullock[6, 7] supported Teilum's theory, and at present Teilum's theory is accepted as valid.

Despite the indication of morphologic similarity of the tumor to the human yolk sac, there have been few comparative studies of the tumor and the human yolk sac. In the report by Gonzalez-Crussi and Roth,[8] it is difficult to detect by light microscopy in the human yolk sac the structure of the placenta of rats for which the term "endodermal sinus tumor" was proposed by Teilum. This may be due to the insufficient number of cases used in previous reports and the fact that until now no studies on the yolk sac in various stages of pregnancy have appeared in the literature. However, our study of yolk sacs in 27 cases showed a similarity between the yolk sac endoderm and the yolk sac tumor. By light microscopy both gave positive results in PAS staining after diastase digestion, and they were also α-fetoprotein–positive. The findings of the endodermal tubules and intracellualr tubules seen in the yolk sac endoderm of the 4-week pregnancy cases resembled those of the reticular pattern of the tumor. The structure in which the papillary endoderm contained blood cells in the center and protruded into the endodermal tubules was spherical in the serial sections but closely resembled the Schiller-Duval body. Moreover, an electron microscopic comparison between the yolk sac tumor and the yolk sac endoderm showed morphologic similarities such as a mode of cellular bonding, microvilli, nucleoli-presenting nucleolonema, layered rough endoplasmic reticulum closely adjacent to mitochondria, rosette-forming glycogen, ovoid and amorphous granules, and basement membrane and basement membrane–like materials. As mentioned above, morphologically the tumor had a marked similarity to the yolk sac endoderm.

Gonzalez-Crussi and Roth[8] reported that the morphologic change began at the tenth week of pregnancy. However, in our study the morphologic findings and the functional study of α-fetoprotein production of the yolk sac suggest that activity is present until approximately the seventh week of pregnancy and declines toward degeneration and involution thereafter.

In respect to the basement membrane of the human yolk sac, both Hoyes[12] and Fukuda[13] have reported no observations of the basement membrane when the yolk sac was observed in the eighth and tenth week and in the fourth to fifth week of pregnancy, respectively. However, Nogales-Fernandez et al.[9] have mentioned that when the yolk sac was observed in the seventh and twelfth week, the basement membrane was seen between the endoderm and the mesenchyme in the yolk sac of a 7-week pregnancy, and its presence was the main characteristic of the young yolk sac. We also observed the basement membrane in the yolk sacs of 6- and 7-week pregnancies. This fact, as Nogales-Fernandez et al.[9] have indicated, suggests that the basement

membrane may be main characteristic of the young yolk sac. However, our observations of the yolk sacs of 4- and 5-week pregnancies showed the absence of the basement membrane, as was also reported by Fukuda.[13] These findings suggest that the basement membrane is observable during a certain period, that is, the period of greatest activity in the human yolk sac. The basement membrane is presumably a material such as α-fetoprotein produced in the yolk sac.

In a study of the yolk sac tumor, Abelev et al.[14] have suggested that the α-fetoprotein becomes one index of yolk sac tumors, including extragonadal ones, and Nogales-Fernandez et al.[9] have proved the production of α-fetoprotein by means of the tissue culture method. In our patients also the serum α-fetoprotein was positive, and furthermore, α-fetoprotein was proved to be present in the tumor cells. With regard to human yolk sacs, Gitlin et al.[15] have proved the production of α-fetoprotein by culturing human yolk sacs and reported that the production was especially conspicuous in young yolk sacs. Our demonstration of the localization of α-fetoprotein has disclosed that α-fetoprotein is positive in the yolk sac endoderm until the seventh week of pregnancy and that the production of α-fetoprotein is the most conspicuous in this period. Since this period is nearly coincident with the time of pregnancy at which the basement membrane was seen, the basement membrane presumably consists partly of α-fetoprotein. The electron microscopic findings of the endoderm exhibit vigorous protein synthesis, which supports these facts.

As mentioned above, in view of the morphologic and functional aspects of α-fetoprotein production, it was concluded that the tumor resembles the human yolk sac endoderm before 7 weeks of pregnancy.

REFERENCES

1. Schiller W. Mesonephroma ovarii. Cancer 1939;35:1-21.
2. Teilum G. Endodermal sinus tumors of the ovary and testis. Comparative morphogenesis of the so-called masonephroma ovarii (Schiller) and extraembryonic (yolk sac–allantoic) structures of the rat's placenta. Cancer 1959;12:1092-105.
3. Duval N. Le placenta des rongeurs. J Anat Physiol (Paris) 1891;27:515-612.
4. Teilum G, Albrechtsen R, Nørgaad-Pedersen B. Immunofluorescent localization of alpha-fetoprotein synthesis in endodermal sinus tumor (yolk sac tumor). Acta Pathol Microbiol Scand [A] 1977;82:586-8.
5. Teilum G, Albrechtsen R, Nørgaard-Pedersen B. The histogenetic embryologic basis for reappearance of alpha-fetoprotein in endodermal sinus tumors (yolk sac tumors) and teratomas. Acta Pathol Microbiol Scand [A] 1975; 83:80-6.
6. Huntington RW Jr, Bullock WK. Yolk sac tumors of the ovary. Cancer 1970;25:1357-67.
7. Huntington RW Jr, Bullock WK. Yolk sac tumors of extragonadal origin. Cancer 1970;25:1368-76.
8. Gonzalez-Crussi F, Roth LM. The human yolk sac and yolk sac carcinoma: an ultrastructural study. Hum Pathol 1976;7:675-91.
9. Nogales-Fernandez F, Silverberg SG, Bloustein PA, et al. Yolk sac carcinoma (endodermal sinus tumor): ultrastructure and histogenesis of gonadal and extragonadal tumors in comparision with normal human yolk sac. Cancer 1977;39:1462-74.
10. Sternberger LA, Hanker JS, Donati ED, et al. Method for enhancement of electron microscopic visualization of embedded antigen by bridging osmium to uranium antibody with thiocarbohydrazide. J Histochem Cytochem 1966;14:711-8.
11. Sternberger LA. Electron microscopic immunocytochemistry: a review. J Histochem Cytochem 1967;15:139-59.
12. Hoyes AD. The human fetal yolk sac: an ultrastructural study of 4 specimens. Z Zellforsch 1969;99:469-90.
13. Fukuda T. Fetal hemopoiesis I. Electron microscopic studies on human yolk sac hemopoiesis. Virchows Arch [Zellpathol] 1973;14:197-213.
14. Abelev GI, Assecritova IV, Kraevsdy NA, et al. Embryonal serum α-globulin in cancer patients: diagnostic value. Int J Cancer 1967;2:551-8.
15. Gitlin D, Perricelli A. Synthesis of serum albumin, prealbumin, α-fetoprotein, α_1-antitrypsin and transferrin by human yolk sac. Nature 1970;228:995-7.

Effects of maternal anemia on uteroplacental and fetal oxidative metabolism in sheep

Mary E. Paulone, M.D., Daniel I. Edelstone, M.D., and Ann Shedd, M.D.
Pittsburgh, Pennsylvania

We studied the effects of acute isovolemic maternal anemia on several variables of uterine and fetal oxygenation to answer these two questions: (1) Does maternal anemia cause reductions in oxygen delivery to the uterus, placenta, and fetus? (2) If so, what is the impact of such reductions on fetal oxidative metabolism? In 15 chronically catheterized pregnant sheep and fetal lambs, we measured uterine and umbilical blood flows and uterine and fetal oxygen deliveries, oxygen extractions, and oxygen consumptions at various, randomly selected maternal hematocrits ranging from 30% to 8%. We altered maternal hematocrit by performing isovolemic exchange transfusions with plasma or packed red blood cells. Uterine and umbilical blood flows were measured with the radionuclide-labeled microsphere technique, and the variables of oxygenation were calculated with modifications of the Fick principle. Decreases in maternal hematocrit led to linear reductions in oxygen delivery to the uterus, placenta, and fetus. Despite reductions in oxygen delivery of up to 50%, fetal oxygen consumption was maintained, because of compensatory increases in oxygen extraction. When hematocrit (and thus fetal oxygen delivery) was reduced by more than 50%, fetal oxygen consumption and the arterial blood base excess both decreased, indicating that, at these hematocrit levels, the supply of oxygen to fetal tissues was inadequate for the demands for oxygen by these tissues. (Am J Obstet Gynecol 1987;156:230-6.)

Key words: Uterine blood flow, umbilical blood flow, fetal oxygen consumption, acute isovolemic anemia

Anemia during pregnancy is a common clinical problem, affecting up to 5% of all pregnancies.[1] Anemic women are more likely to be delivered of infants who are stillborn or hypoxic at birth. Although the adverse consequences of maternal anemia have been known for many years, the specific reasons for these poor outcomes are unknown. During anemia the delivery of oxygen to the fetus may be reduced. The ways in which uteroplacental or fetal tissues could adjust to a reduced oxygen delivery should be the same as those by which tissues in nonpregnant animals compensate for anemia.[2] Although the cardiovascular effects of anemia on nonpregnant animals have been systematically studied,[2, 3] there are few experimental data on the circulatory consequences of anemia when it occurs during pregnancy.[4, 5] The purpose of our studies was to answer the following two questions: (1) Does maternal anemia cause reductions in oxygen supply to the uterus, placenta, and fetus? (2) If so, what is the impact of these reductions on fetal oxidative metabolism? To answer these questions, we have designed experiments in pregnant sheep to assess the effects of variations in maternal hematocrit on the oxygen tensions and oxygen concentrations of uterine and umbilical blood, the oxygen consumptions of the uterus and fetus, and the acid-base balance of fetal blood.

From the Department of Obstetrics and Gynecology, University of Pittsburgh School of Medicine, Magee-Womens Hospital.
This work was supported by grants from the National Institutes of Health (HD 16368) and from the Magee-Womens Hospital Research Fund.
This work was presented in part at the Thirty-first Annual Meeting of the Society for Gynecologic Investigation, San Francisco, California, March 21-24, 1984, and at the Sixty-eighth Annual Meeting of the Federation of American Societies for Experimental Biology, St. Louis, Missouri, April, 1984. An abstract appeared in Fed Proc 1984;43:332.
Received for publication May 20, 1986; revised August 6, 1986; accepted August 19, 1986.
Reprint requests: Daniel I. Edelstone, M.D., Department of Obstetrics and Gynecology, Magee-Womens Hospital, Pittsburgh, PA 15213.

Methods

Preparation of animals. We surgically prepared 15 singleton pregnant sheep whose gestational ages ranged from 105 to 130 days (term = 147 days).[6] After an epidural or spinal anesthetic (tetracaine hydrochloride, 1%) was administered to the mother, we inserted catheters into two maternal hind limb arteries and a vein in all 15 sheep. These catheters were advanced into the descending aorta and inferior vena cava, respectively. In seven of the 15 sheep, a catheter was threaded retrograde into the maternal left ventricle by way of a carotid artery. Correct location of this catheter tip was established by observing the characteristic left ventricular pressure waveform. In 11 of the sheep, we opened the maternal abdomen and inserted a catheter into the uterine vein draining the horn containing the

fetus; the tip of this catheter was placed approximately 4 cm caudad to the ovary.[8] In 10 of the sheep, we incised the pregnant uterine horn and inserted catheters into two fetal hind limb arteries and a vein.[6, 7] These catheters were advanced into the fetal descending aorta and abdominal inferior vena cava, respectively. A catheter was also placed into the common umbilical vein through a periumbilical skin incision.[6] Anesthesia for catheter placements in the fetus was provided by local infiltration with lidocaine hydrochloride, 0.5%. All incisions were closed and catheters were led subcutaneously to the ewe's flank where they were stored in a pouch. The animals recovered for 2 to 7 days before any experiments were performed. We studied the unanesthetized mothers and fetuses as the ewes stood quietly in an open transport cage.

Experimental procedures. We measured uterine and umbilical blood flows, uterine and fetal oxygen deliveries, oxygen extractions, and oxygen consumptions and fetal acid-base balance at various, randomly determined levels of maternal hematocrit ranging from 30% to 8%. From the 15 animals studied, we obtained data on the uterine blood flow and uterine oxidative responses to maternal anemia (uterine responses) in seven of the sheep and on umbilical blood flow and fetal oxidative responses (fetal responses) in 10 of the sheep. To produce maternal anemia of varying degrees, we performed isovolemic exchange transfusions by infusing plasma or packed red blood cells, obtained from donor pregnant sheep, through one maternal catheter while withdrawing whole blood at the same rate from a second catheter. Hematocrits were changed slowly during 1 to 4 hours, during which time maternal and fetal aortic blood pressures (obtained with Statham P23Db pressure transducers) and heart rates (via cardiotachometers) were continuously monitored and recorded (Beckman 611A polygraph).[6] As the exchange transfusions were progressing, we periodically obtained blood samples for the determination of maternal hematocrit. When hematocrit had reached a previously selected level, we discontinued the exchange transfusion and waited for maternal hematocrit and maternal and fetal heart rates and blood pressures to be stable for at least 30 minutes.

In the studies on the uterine responses to maternal anemia, we withdrew 0.6 ml blood samples simultaneously from the maternal descending aorta and uterine vein at each hematocrit studied. Then approximately 5 to 10 × 10^6 radionuclide-labeled microspheres (15 μm diameter) were injected into the maternal left ventricle, while a reference blood sample was withdrawn from the descending aorta at 7.9 ml/min.[9] We used this high withdrawal rate during the microsphere injections to ensure that blood flow measurements made in the anemic animals were accurate.[10] In the studies on the fetal responses to maternal anemia, we obtained 0.6 ml blood samples simultaneously from the fetal descending aorta and umbilical vein, after which 1 to 2 × 10^6 microspheres were injected into the fetal inferior vena cava, while a reference blood sample was withdrawn from the fetal descending aorta at 3.9 ml/min.[6, 7] The volume of fetal blood withdrawn was replaced with an equivalent amount of fresh maternal whole blood of similar hematocrit so that fetal blood volume could be kept constant. Maternal and fetal aortic blood pressures and heart rates were stable before, during, and after microsphere injections.[9] All blood samples were analyzed for blood gases and pH, hematocrit, and blood oxygen concentration.[6, 7] Blood samples were kept on ice and were analyzed within 15 minutes. Blood gases and pH were measured at 37° C with standard electrodes and a blood gas meter (Instruments Laboratories) and were then corrected to 39° C for maternal blood samples and 39.5° C for fetal blood samples; hematocrit was measured in duplicate with the microcapillary technique; and blood oxygen concentration was measured with an oxygen analyzer (Lex-O_2-Con, Lexington Instruments). When the blood sampling and blood flow measurements were completed at any given maternal hematocrit, we changed hematocrit to a new level and repeated the measurements. For the uterine responses to maternal anemia, we made 27 observations in seven sheep; for the fetal responses, we made 33 observations in 10 sheep.

Preparation of tissues and calculation and analysis of data. At the end of the study, the sheep and fetus were killed with an overdose of pentobarbital sodium, and all catheter locations were verified visually. The uterus, placental cotyledons, placental membranes, and fetus were removed and weighed. Uterine, placental, and membrane tissues were incinerated at 280° C for 48 hours, after which the ashed tissues were packed into counting vials. The amounts of each radionuclide in the tissues and in the aortic blood reference samples were measured with a well-type gamma scintillation counter (Model 1185R, Tracor) and a 1024-channel pulse-height analyzer (Ultima II, Norland Corp).[9] Nuclide isotope separation was performed by computer (Nova 3, Data General). Aortic blood reference samples and all tissue samples contained more than 1500 microspheres to ensure that the error in any blood flow measurement was <5% at the 95% confidence limits.[9]

We computed uterine ($\dot{Q}_{ut}$) and umbilical ($\dot{Q}_{umb}$) blood flows as follows:

$$\dot{Q}_{ut} = \left(\frac{CPM_{ut}}{CPM_{ref\text{-}M}}\right)\dot{Q}_{ref\text{-}M}$$

$$\dot{Q}_{umb} = \left(\frac{CPM_{umb}}{CPM_{ref\text{-}F}}\right)\dot{Q}_{ref\text{-}F}$$

where CPM_{ut} = radioactive counts per minute in uter-

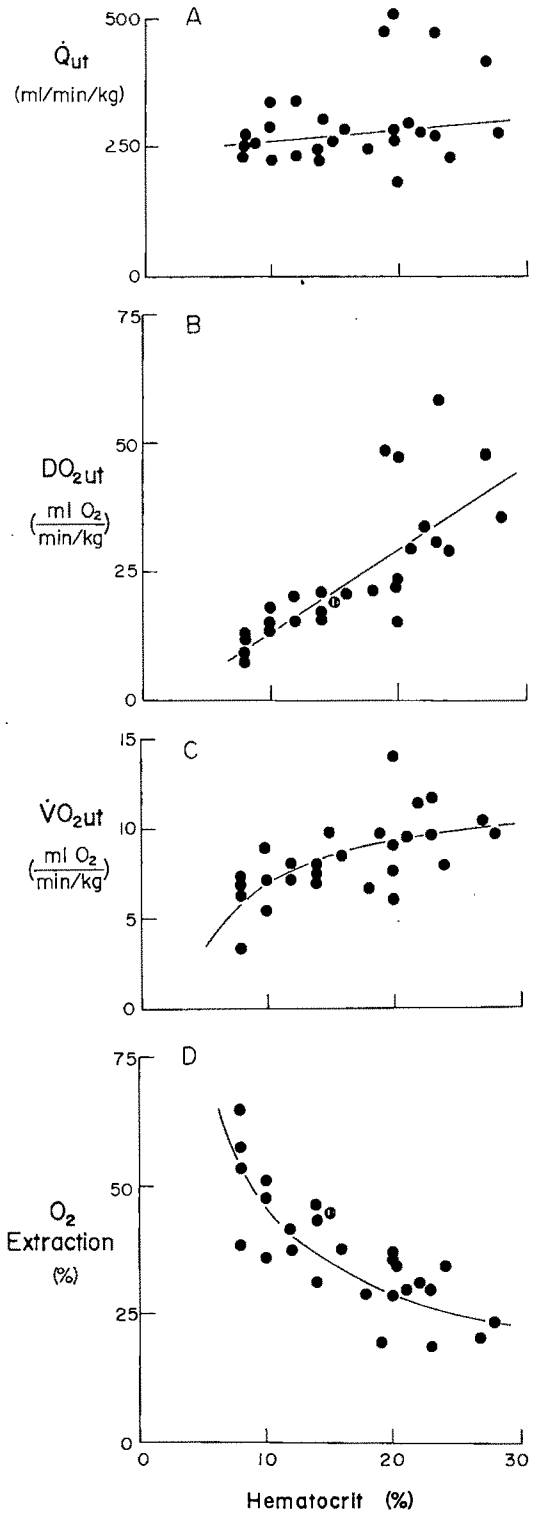


Fig. 1. Uterine blood flow ($\dot{Q}_{ut}$), uterine oxygen delivery (DO_{2ut}), uterine oxygen consumption ($\dot{V}O_{2ut}$), and uterine oxygen extraction as functions of maternal hematocrit. $\dot{Q}_{ut}$, DO_{2ut}, and $\dot{V}O_{2ut}$ are expressed per kilogram of uterus plus placenta plus fetus. *A*, $\dot{Q}_{ut}$ versus hematocrit. y = 235 + 2.7x; r = 0.21, ns. *B*, DO_{2ut} versus hematocrit. y = −4.5 + 1.73x; r = 0.78, $p < 0.001$. *C*, $\dot{V}O_{2ut}$ versus hematocrit. y = −2.2 + 3.9 lnx; r = 0.68, $p < 0.001$. *D*, Oxygen extraction versus hematocrit. $y = 194x^{-0.62}$; r = 0.79, $p < 0.001$. Analyses are based on 27 observations made in seven sheep.

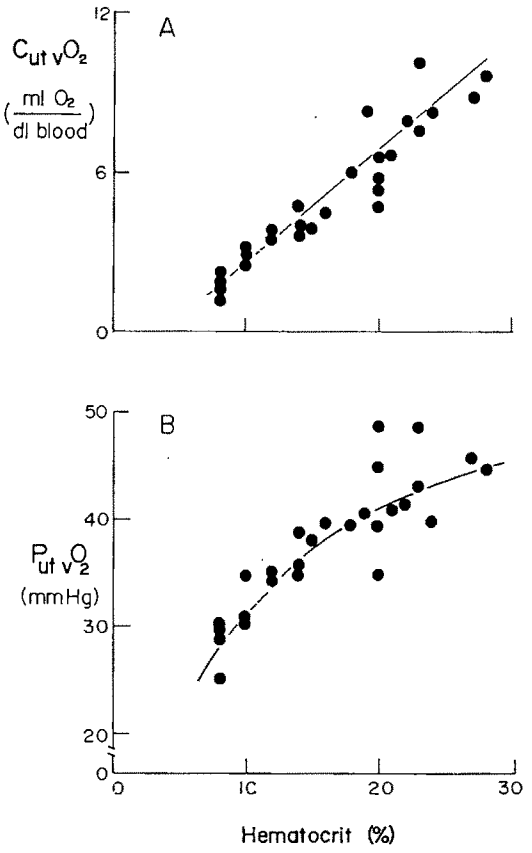


Fig. 2. Uterine venous blood oxygen concentration ($C_{utv}O_2$) and uterine venous blood oxygen tension ($P_{utv}O_2$) as functions of maternal hematocrit. *A*, $C_{utv}O_2$ versus hematocrit. y = −1.4 + 0.4x; r = 0.95, $p < 0.001$. *B*, $P_{utv}O_2$ versus hematocrit. y = 0.3 + 13.6 lnx; r = 0.91, $p < 0.001$. Analyses are based on 27 observations made in seven sheep.

ine muscle plus maternal portion of the placenta and $CPM_{ref\text{-}M}$ = radioactive counts per minute in the maternal aortic blood reference sample, both derived from a maternal injection of microspheres; CPM_{umb} = radioactive counts per minute in the fetal portion of the placenta plus the amniotic and allantoic membranes and $CPM_{ref\text{-}F}$ = radioactive counts per minute in the fetal aortic blood reference sample, both derived from an injection of microspheres into the fetal inferior vena cava; $Q_{ref\text{-}M}$ = maternal descending aortic reference blood flow; and $Q_{ref\text{-}F}$ = fetal descending aortic reference blood flow, determined by calibration of the withdrawal pumps.

Three variables of uterine oxygenation were computed with modifications of the Fick principle[8]:

$$\text{Uterine oxygen delivery } (DO_{2ut}) = \dot{Q}_{ut}\,(CaO_2)$$

$$\text{Uterine oxygen consumption } (\dot{V}O_{2ut}) = \dot{Q}_{ut}\,(CaO_2 - CutvO_2)$$

$$\text{Uterine oxygen extraction} = \dot{V}O_{2ut}/DO_{2ut} = (CaO_2 - CutvO_2)/CaO_2$$

where CaO_2 = oxygen concentration in maternal arterial blood, $CutvO_2$ = oxygen concentration in uterine venous blood, and $\dot{Q}_{ut}$ = uterine blood flow. $\dot{Q}_{ut}$, DO_{2ut}, and $\dot{V}O_{2ut}$ were expressed per kilogram of uterus + placenta + fetus.

We calculated three variables of fetal oxygenation, using the following formulas[6-8]:

$$\text{Fetal oxygen delivery } (DO_{2\ fetus}) = \dot{Q}_{umb}\ (CuvO_2)$$

$$\text{Fetal oxygen consumption } (\dot{V}O_{2\ fetus}) = \dot{Q}_{umb}\ (CuvO_2 - CuaO_2)$$

$$\text{Fetal oxygen extraction} = \dot{V}O_{2\ fetus}/DO_{2\ fetus} = (CuvO_2 - CuaO_2)/CuvO_2$$

where $CuvO_2$ = oxygen concentration in umbilical venous blood, $CuaO_2$ = oxygen concentration in fetal descending aortic (umbilical arterial) blood, and $\dot{Q}_{umb}$ = umbilical blood flow. $\dot{Q}_{umb}$, $DO_{2\ fetus}$, and $\dot{V}O_{2\ fetus}$ were expressed per kilogram of fetus.

Uterine and umbilical blood flows, arterial and venous blood oxygen concentrations and oxygen tensions, and uterine and fetal oxygen deliveries, oxygen consumptions, and oxygen extractions (dependent variables) were plotted against maternal hematocrit (independent variable). We analyzed the data statistically with a weighted least-squares method based on analysis of covariance, with which we could generate regression lines that took into account responses seen in individual animals.[11] With this method of analysis, we could define mathematically the relationships that existed between the various dependent variables and the independent variable. Linear, logarithmic, and power functions were all tested for best fit; the function selected as best describing each relationship had the greatest correlation coefficient associated with the least residual sum of squares.[11] For all analyses, p values <0.01 were used to indicate statistically significant correlations.

Results

In the 15 animals studied, gestational age was 122 ± 5 (mean ± SD) days on the day of study, maternal weight was 43 ± 6 kg, and fetal weight was 2.6 ± 0.4 kg. Maternal hematocrit before the start of the studies was 27% ± 2%. The data are presented in six figures, which illustrate the effects of maternal anemia on uterine blood flow and uterine oxygenation, the transfer of oxygen from uterine blood to umbilical blood, and the delivery of oxygen to and the consumption of oxygen by the fetus.

Uterine blood flow (Fig. 1, *A*) was independent of maternal hematocrit over the range of hematocrits studied, and therefore uterine oxygen delivery decreased linearly as a function of decreasing maternal hematocrit (Fig. 1, *B*). Despite large reductions in uterine oxygen delivery, oxygen consumption by the uterus and its contents, the placenta and fetus, decreased only slightly as hematocrit was reduced to about 15% (Fig. 1, *C*), because oxygen extraction by these tissues increased (Fig. 1, *D*). The increase in uterine oxygen extraction was reflected in decreases in uterine venous blood oxygen concentration and oxygen tension (P_{O_2}) (Fig. 2, *A* and *B*). Furthermore, the decrease in uterine venous blood P_{O_2} was associated with equivalent decreases in umbilical venous blood P_{O_2} (Fig. 3). Umbilical venous blood P_{O_2} was about 14 mm Hg less than uterine venous blood P_{O_2} over the range of maternal hematocrits studied (30% to 8%).

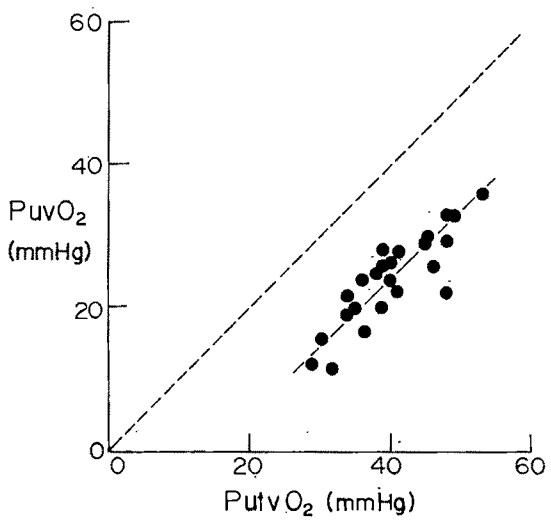


Fig. 3. Relationship of umbilical venous blood oxygen tension ($P_{uv}O_2$) to uterine venous blood oxygen tension ($P_{ut}O_2$) during acute maternal anemia. $y = -14.2 + 0.98x$; $r = 0.85$, $p < 0.001$. Analysis is based on 24 observations made in six sheep. The *dashed line* represents the line of identity.

The reductions in umbilical venous blood P_{O_2} resulting from maternal anemia led to reductions in umbilical venous blood oxygen concentration (Fig. 4, *A*). Umbilical blood flow, however, was unaffected by any level of maternal anemia produced (Fig. 4, *B*). Thus fetal oxygen delivery fell as a direct function of maternal hematocrit (Fig. 4, *C*). In response to this fall in fetal oxygen delivery, oxygen extraction (Fig. 5, *A*) increased sufficiently for fetal oxygen consumption to be maintained despite reductions in maternal hematocrit from 30% to about 15% (Fig. 5, *B*). When hematocrit was reduced to levels below about 15%, fetal oxygen consumption fell, because the further increases in oxygen extraction were insufficient to fully compensate for the further declines in fetal oxygen delivery. Since fetal oxygen extraction = $(CuvO_2 - CuaO_2)CuvO_2$, it follows that $CuaO_2 = CuvO_2$ (1 − fetal oxygen extraction). Thus increases in fetal oxygen extraction were

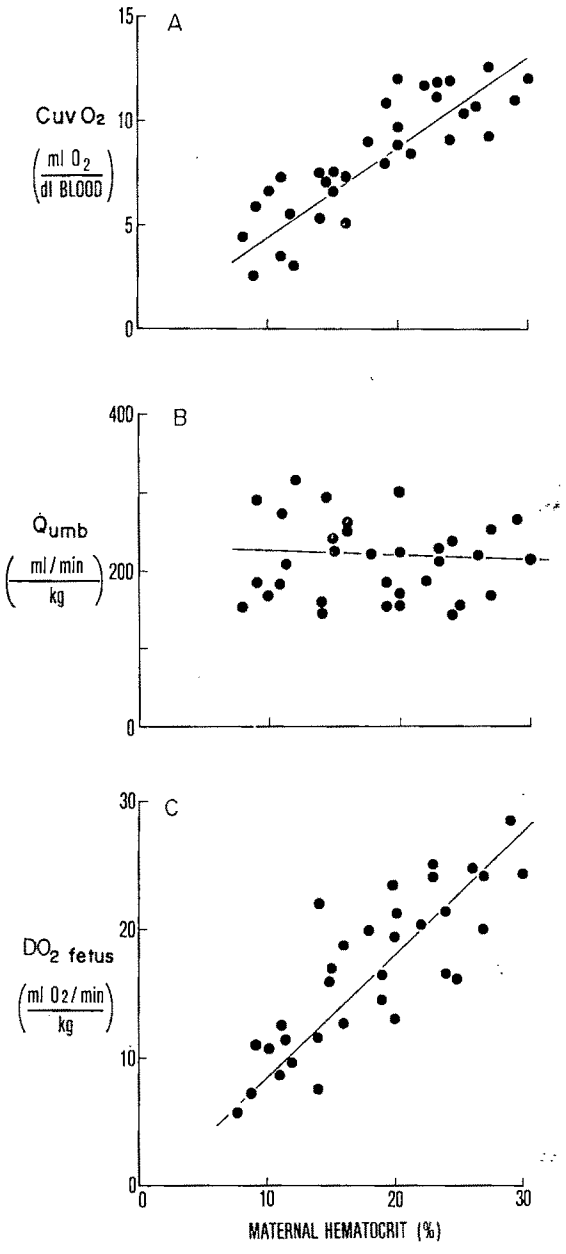


Fig. 4. Umbilical venous blood oxygen concentration ($C_{uv}O_2$), umbilical blood flow ($\dot{Q}_{umb}$), and fetal oxygen delivery ($DO_{2\,fetus}$) as functions of maternal hematocrit. $\dot{Q}_{umb}$ and $DO_{2\,fetus}$ are expressed per kilogram of fetus. *A*, $CuvO_2$ versus hematocrit. $y = 0.24 + 0.44x$; $r = 0.84$, $p < 0.001$. *B*, $\dot{Q}_{umb}$ versus hematocrit. $y = 224 - 0.66x$; $r = 0.08$, NS. *C*, $DO_{2\,fetus}$ versus hematocrit. $y = -0.15 + 0.94x$; $r = 0.81$, $p < 0.001$. Analyses are based on 33 observations made in 10 sheep.

associated with reductions in fetal arterial blood oxygen concentration: Arterial blood oxygen concentration was linearly related to maternal hematocrit ($y = -1.38 + 0.36x$; $r = 0.90$, $p < 0.001$).

Because maternal anemia led to reductions in fetal oxygen delivery and arterial blood oxygen concentration, we were also interested in identifying how thes reductions affected fetal acid-base balance. Fig. 6 show that, as long as oxygen delivery to the fetus was greate than approximately 14 ml O_2/min/kg of fetus, whic corresponded to maternal hematocrits greater tha about 15%, fetal oxygen consumption and arteri blood base excess remained within normal limits. Hov ever, when fetal oxygen delivery was ≤14 ml/min/k of fetus (maternal hematocrit ≤15%), oxygen cor sumption and arterial blood base excess both fell, ir dicating that, at these severities of maternal anemi delivery of oxygen to the fetus was inadequate for th demands for oxygen by the fetus.

Comment

The data in this report establish that acute matern anemia causes reductions in the delivery of oxygen t the uterus, placenta, and fetus. Despite such reductior in oxygen delivery, fetal oxidative metabolism can b sustained over a wide range of maternal hematocrit In our studies anemia had no significant effect on ute ine blood flow. Uterine perfusion could have change as a consequence of direct rheologic effects of the an mia on blood viscosity[2] or as a result of secondary effec of anemia on neural or endocrine vascular regulation It is possible that both of these factors were affecte equally but in opposite directions, with the net resu being that uterine blood flow did not change. Sinc anemia was associated with reductions in arterial bloo oxygen concentration, the delivery of oxygen to th uterus (uterine blood flow × arterial blood oxyge concentration) decreased linearly as a function of m ternal hematocrit. Regardless of this decrease, oxyge consumption by the uterus and its contents, the pl centa and fetus, was only minimally affected by redu tions in hematocrit, and thus in oxygen delivery, of a much as 50% below normal levels. Wilkening an Meschia[8] noted similar responses when uterine oxyge delivery was reduced ≤50% as a result of decreases i uterine blood flow. In our studies uterine oxygen cor sumption was maintained at or near normal levels whe maternal hematocrits were greater than about 15% because oxygen extraction increased as compensatio Oxygen extraction is a measure of two diffusion var ables, the capillary-to-cell P_{O_2} gradient and the capillar surface area (capillary density) available for oxygen e change.[13] The increase in uterine oxygen extractio that we observed during acute anemia could have r sulted from an increase in the P_{O_2} gradient betwee maternal-placental and fetal-placental capillaries c from an increase in the density of maternal or fet placental capillaries or both. Our data do not indica which of these diffusion variables was more likely to b affected by maternal anemia.

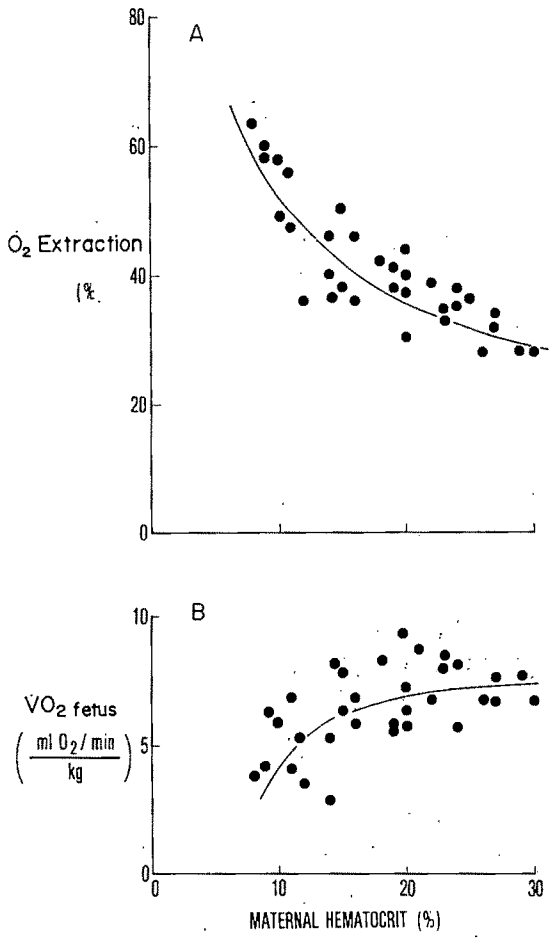


Fig. 5. Relationship of fetal oxygen extraction and fetal oxygen consumption ($\dot{V}O_{2\,fetus}$) to maternal hematocrit. $\dot{V}O_{2\,fetus}$ is expressed per kilogram of fetus. *A*, Oxygen extraction versus hematocrit. $y = .165x^{-0.50}$; $r = 0.86$, $p < 0.001$. *B*, $\dot{V}O_{2\,fetus}$ versus hematocrit. $y = (27.2\ \ln x - 50.6)/\sqrt{x}$; $r = 0.69$, $p < 0.001$. Data analyses are based on 33 observations made in 10 sheep.

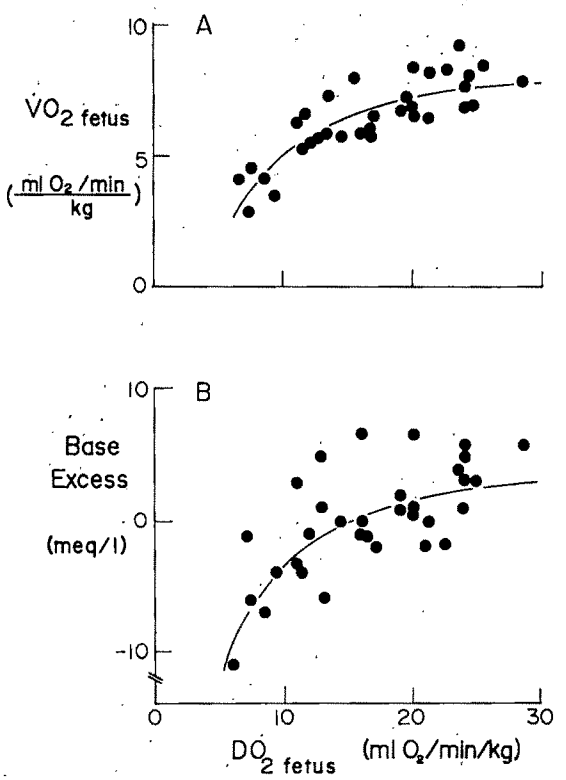


Fig. 6. Fetal oxygen consumption ($\dot{V}O_{2\,fetus}$) and fetal arterial blood base excess as functions of fetal oxygen delivery ($DO_{2\,fetus}$). $\dot{V}O_{2\,fetus}$ and $DO_{2\,fetus}$ are expressed per kilogram of fetus. *A*, $\dot{V}O_{2\,fetus}$ versus $DO_{2\,fetus}$. $y = -3.99 + 3.74\ \ln x$; $r = 0.84$, $p < 0.001$. *B*, Base excess versus $DO_{2\,fetus}$. $y = -19.9 + 7.29\ \ln x$; $r = 0.69$, $p < 0.001$. Data analyses are based on 33 observations made in 10 sheep.

The increase in uterine oxygen extraction we noted was reflected in reductions in uterine venous blood oxygen concentration and P_{O_2}. Bissonette et al.[4] observed similar responses to anemia in experiments on anemic pregnant goats. Such reductions in uterine venous blood P_{O_2} have important implications insofar as the transfer of oxygen from mother to fetus via the placenta is concerned.[14] The placentas of several species, including sheep and man, function as concurrent exchangers for oxygen. In a concurrent gas exchanger, the P_{O_2} of the blood in the donor effluent (maternal-placental venous blood, approximated by measuring P_{O_2} in uterine venous blood) must always equal or exceed the P_{O_2} of the blood in the recipient effluent (fetal-placental venous blood, approximated by measuring P_{O_2} in umbilical venous blood).[14] Our data showed that uterine venous blood P_{O_2} exceeded umbilical venous blood P_{O_2} by about 14 mm Hg over the range of maternal hematocrits studied, 30% to 8%. This relationship is similar to that observed by Wilkening and Meschia[8] and by Rankin et al.,[15] who used widely differing methods to reduce uterine venous blood P_{O_2}. Many factors could have contributed to this uterine venous-umbilical venous blood P_{O_2} difference, including uneven perfusion within the maternal-placental and fetal-placental circulations, differing hemoglobin types and hemoglobin-oxygen affinities in the maternal and fetal bloodstreams, shunting of uterine and umbilical blood flows away from placental gas exchange surfaces, and the high rate of oxygen consumption by the placenta itself.[8, 14] Although any or all of these factors could have been affected by acute maternal anemia, our data indicate that, overall, anemia had no effect on the function of the placenta as an oxygen exchanger.

Because umbilical venous and uterine venous blood P_{O_2} values were closely linked during acute maternal anemia, umbilical venous blood oxygen concentration was directly related to maternal hematocrit. Since um-

bilical blood flow was unaffected by any level of anemia produced, our studies indicate that oxygen delivery to the fetus (umbilical venous blood oxygen concentration × umbilical blood flow) was a linear function of maternal hematocrit. However, despite reductions in maternal hematocrit and thus in fetal oxygen delivery, of up to about 50%, fetal oxygen consumption was kept constant as a result of compensatory increases in fetal oxygen extraction.[6-8] As a consequence of this greater oxygen extraction, fetal arterial blood oxygen concentration was reduced. Cefalo et al.[5] also observed hypoxemia in fetal lambs whose mothers were made anemic acutely. Since oxygen supply to fetal tissues is a function of the arterial blood oxygen concentration and the tissue blood flow, fetal hypoxemia induced by maternal anemia will be associated with decreases in oxygen delivery to some fetal organs and tissues. When these decreases are severe enough, tissue anaerobic metabolism will result.[13]

The stable fetal oxygen consumption we observed during reductions in fetal oxygen delivery of up to 50% was not due to an improvement in the efficiency of placental gas exchange during maternal anemia (see Fig. 3).[8] Rather it was an indication that, under normal conditions, the delivery of oxygen to the fetus is considerably in excess of the demands for oxygen by the fetus (see Fig. 6). Reductions in fetal oxygen delivery from about 28 to about 14 ml O_2/min/kg of fetus were not associated with significant decreases in either fetal oxygen consumption or arterial blood base excess. However, when oxygen delivery was decreased to less than about 14 ml O_2/min/kg, both oxygen consumption and base excess fell as oxygen delivery became inadequate for oxygen demands. These responses are qualitatively and quantitatively the same as the responses that have been observed when fetal oxygen delivery has been reduced by other maneuvers, such as reductions in uterine blood flow[8] or umbilical blood flow[7] or the development of fetal anemia.[6] Taken together all of these experiments strongly support the concept that the level of fetal oxygen delivery, rather than the specific condition that may adversely affect oxygen delivery acutely, is more important in predicting the rate of fetal oxidative metabolism.[6, 7]

Our current data cannot and should not be extrapolated to encompass chronic degrees of anemia in animal or human pregnancies. It is unknown how chronic anemia might affect fetal oxygenation. We do, however, have preliminary data from chronically anemic pregnant sheep that indicate that fetal oxygen delivery can be reduced sufficiently to affect not only fetal oxidative metabolism but also fetal growth.

In summary, the data in the present report indica
that acute maternal anemia causes reductions in fet
oxygen delivery, for which the fetus can compensate
ensure that oxygen delivery to fetal tissues is adequa
for oxidative metabolism. When maternal anemia
sufficiently severe, however, the delivery of oxygen
the fetus becomes inadequate for the demands for ox
gen by fetal tissues. If similar responses were to
observed in human pregnancies, these responses migl
help to explain why anemic pregnant women are mo
likely to be delivered of infants who are stillborn
hypoxic at birth.

We wish to thank Anthony Battelli, Pat Moran, an
Lynn Heckman for their excellent technical assistanc

REFERENCES

1. Garn SM, Ridella SA, Petzold AS, Falkner F. Matern hematologic levels and pregnancy outcomes. Semin Pe natol 1981;5:155-62.
2. Fan F-C, Chen RYZ, Schuessler GB, Chien S. Effects hematocrit variations on regional hemodynamics ar oxygen transport in the dog. Am J Physiol 1980;23 H545-52.
3. Shepherd AP, Riedel GL. Optimal hematocrit for ox genation of canine intestine. Circ Res 1982;51:233-40.
4. Bissonnette JM, Metcalfe J, Hohimer AR, Pernoll M Welch JE, Lawson MS. Uterine oxygen uptake in the pre nant pygmy goat. Respir Physiol 1980;42:373-81.
5. Cefalo RC, Seeds JW, Proctor HJ, Baker VV. Matern and fetal effects of exchange transfusion with a red bloc cell substitute. AM J OBSTET GYNECOL 1984;148:859-6
6. Edelstone DI, Caine ME, Fumia FD. Relationship of fet oxygen consumption and acid-base balance to fetal h matocrit. AM J OBSTET GYNECOL 1985;151:844-51.
7. Edelstone DI, Peticca BB, Goldblum LJ. Effects of m ternal O_2 administration on fetal oxygenation during r ductions in umbilical blood flow in fetal lambs. AM J O STET GYNECOL 1985;152:351-8.
8. Wilkening RB, Meschia G. Fetal oxygen uptake, oxyge ation, and acid-base balance as a function of uterine bloc flow. Am J Physiol 1983;244:H749-55.
9. Heymann MA, Payne BD, Hoffman JIE, Rudolph AI Blood flow measurements with radionuclide-labeled pa ticles. Prog Cardiovasc Dis 1977;20:55-79.
10. Rosenberg AA, Jones MD Jr, Koehler RC, Traystman R Lister G. Precautions for measuring blood flow durir anemia with the microsphere technique. Am J Physi 1983;244:H308-11.
11. Zar JH. Biostatistical analysis. Englewood Cliffs, New Je sey: Prentice-Hall, 1974.
12. Greiss FC Jr. A clinical concept of uterine blood flow du ing pregnancy. Obstet Gynecol 1967;30:595-604.
13. Edelstone DI, Holzman IR. Fetal intestinal oxygen co sumption at various levels of oxygenation. Am J Physi 1982;242:H50-4.
14. Meschia G. Physiology of transplacental diffusion. I Wynn R, ed. Obstetrics and gynecology annual. New Yor Appleton-Century-Crofts, 1976.
15. Rankin JHG, Meschia G, Makowski EL, Battaglia FC. R lationship between uterine and umbilical venous PO_2 sheep. Am J Physiol 1971;220:1688-92.

Triiodothyronine receptors in porcine granulosa cells

Najib G. Wakim, M.D., Natarajan Ramani, Ph.D., and Ch. V. Rao, Ph.D.
Louisville, Kentucky

Triiodothyronine has been found to enhance gonadotropin- and insulin-stimulated morphologic luteinization and progesterone production by porcine granulosa cells in culture. The role of triiodothyronine in the above events is not well defined. One possibility is that triiodothyronine effects are direct and are receptor mediated. To test this concept, we undertook this study to investigate the presence of triiodothyronine receptors in granulosa cells aspirated from large (6 to 12 mm), medium (3 to 5 mm), and small (1 to 2 mm) porcine ovarian follicles. Crude nuclei were isolated and tested for iodine 125–triiodothyronine specific binding. Binding was time and temperature dependent and maximal in cells from small follicles at pH 8. Scatchard analysis yielded a mean apparent dissociation constant of 5.5×10^{-9} mol/L and a mean apparent total number of binding sites of 1.0 pmol/mg of deoxyribonucleic acid. Competition experiments revealed the following relative binding affinities: triiodothyronine > L-thyroxine > reverse triiodothyronine. Gonadotropins, prostaglandins, epidermal growth factor, and insulin did not compete with ^{125}I-triiodothyronine for binding. We conclude that there are nuclear binding sites in porcine granulosa cells with characteristics expected of a triiodothyronine receptor which might mediate a direct action of triiodothyronine on granulosa cells. (Am J Obstet Gynecol 1987;156:237-40.)

Key words: Triiodothyronine receptor, granulosa cells

Nongonadotropic hormones, specifically insulin, cortisol, and thyroid hormones, have been found to affect luteinization and growth of granulosa cells in vitro in humans,[1-2] as well as in animals.[3-4] Channing et al.[4] found that insulin, cortisol, and thyroxine acted synergistically with luteinizing hormone and follicle-stimulating hormone in cultured porcine granulosa cells to enhance morphologic luteinization and progestin secretion.[4] They also found that insulin, thyroxine, and cortisol increased luteinizing hormone/human chorionic gonadotropin receptors in porcine granulosa cell cultures exposed to follicle-stimulating hormone. Thus it appears that thyroid hormones play a direct effect on granulosa cells in vitro. This role may be receptor mediated. The presence of triiodothyronine receptors in granulosa cells has never been described as far as we know. It is the purpose of this paper to demonstrate the existence of triiodothyronine binding sites in porcine granulosa cells.

Material and methods

Reagents. Unlabeled triiodothyronine and reverse triiodothyronine were purchased from Calbiochem (San Diego, California), thyroxine was obtained from Sigma Chemical Company (St. Louis, Missouri), and ^{125}I-triiodothyronine with a specific activity of 135 to 165 μCi/μg was purchased from New England Nuclear (Boston, Massachusetts). All other reagents were of the highest grade that is commercially available.

Harvesting of follicles. Porcine ovaries were obtained from freshly slaughtered pigs in a local meat packing plant. The ovaries were placed in 0.9% sodium chloride solution and transported to the laboratory on ice. Granulosa cells were harvested from small (1 to 3 mm), medium (3 to 5 mm), and large (6 to 12 mm) follicles immediately on arrival in the laboratory with the use of a 27-gauge needle and a tuberculin syringe. In the case of medium and large follicles the follicles were reflushed repeatedly with their follicular fluid during the same puncture.

Isolation of nuclei. Nuclei were extracted with salt according to a slight modification of a method described previously.[1] The follicular fluid was kept on ice while being harvested from the follicles and was centrifuged immediately after collection at 500 gm for 5 minutes at 4° C. The pellet was suspended in homogenizing buffer, pH 7.6, containing 10 mmol of Tris-hydrochloride, 3 mmol of calcium chloride, 1 mmol of dithiothreitol, and 0.25 mol of sucrose and then homogenized with use of a Teflon glass homogenizer with 12 strokes. The homogenate was centrifuged at 800 gm for 10 minutes at 4° C. Part of the crude nuclear preparation was used for deoxyribonucleic acid content estimation by means of the diphenylamine reaction with use of calf thymus deoxyribonucleic acid as the stan-

From the Department of Obstetrics and Gynecology, University of Louisville.
Received for publication June 26, 1986; revised and accepted August 29, 1986.
Reprint requests: Najib G. Wakim, M.D., Department of Obstetrics and Gynecology, Medical College of Ohio, C. S. #10008, Toledo, OH 43699.

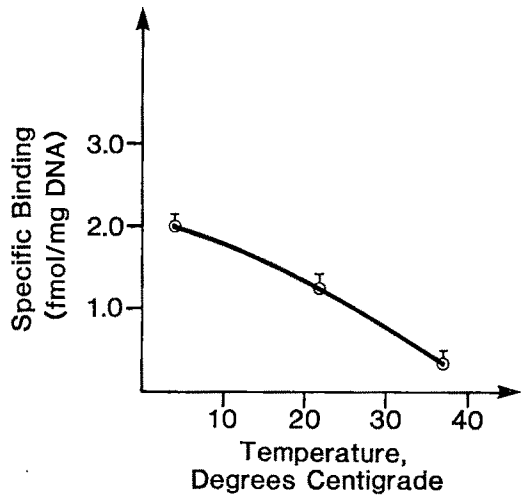


Fig. 1. Temperature dependence of ^{125}I-triiodothyronine binding by granulosa cell nuclei after 3 hours of incubation at 4° C, 22° C, and 37° C. Each assay was performed in triplicate. The *vertical bars* indicate the SEM.

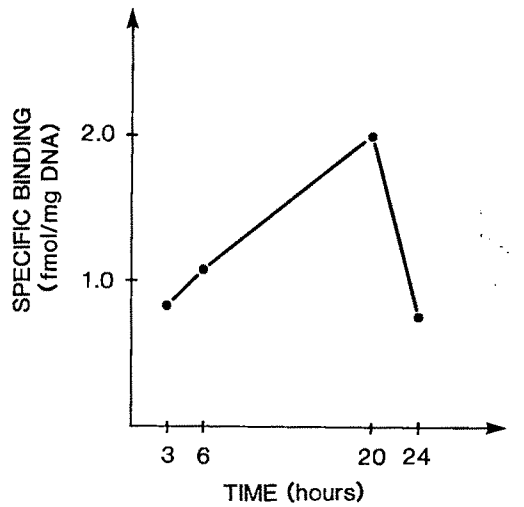


Fig. 2. ^{125}I-triiodothyronine binding as a function of time. Nuclei were isolated from granulosa cells obtained from small (1 to 2 mm) follicles as described. Aliquots of nuclear suspensions containing 14 to 15 μg of deoxyribonucleic acid were incubated in triplicate at 4° C for the indicated time periods.

dard.[5] The rest of the homogenate pellet was resuspended in the assay buffer.

Triiodothyronine binding assays. All experiments were performed on the same day of the collection of the porcine ovaries. Neither ovaries nor follicular fluid were frozen overnight. The triiodothyronine binding studies were performed by addition of 0.2 ml of the nuclear suspension (containing 14 to 15 μg of deoxyribonucleic acid in all experiments) to 12 by 75 mm tubes containing ^{125}I-triiodothyronine (at the concentrations indicated in individual experiments) in 0.2 ml of the assay buffer. A thousandfold excess of unlabeled

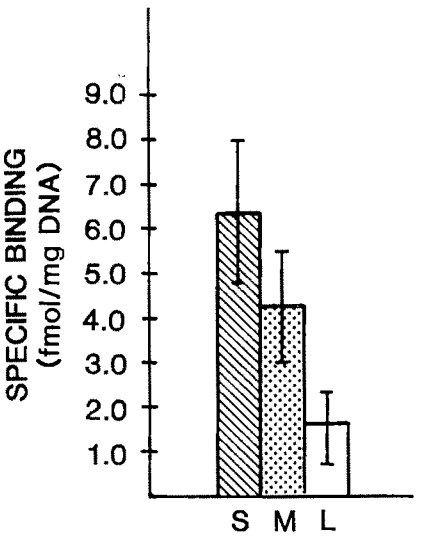


Fig. 3. ^{125}I-triiodothyronine binding to granulosa cells from porcine follicles of different sizes. *S* = Small follicles (1 to 2 mm), *M* = medium (3 to 5 mm), and *L* = large (6 to 12 mm). Mean ± 1 SD is shown. Each assay was performed in triplicate. There was a significant difference in binding between granulosa cells from small follicles and large follicles ($p < 0.01$).

triiodothyronine was used to assess nonspecific binding in parallel samples. After completion of incubations, 1 ml of assay buffer was added to each tube. The tubes were then centrifuged at 3000 gm for 20 minutes at 4° C. The supernatant was discarded, and the pellets were counted in a Beckman Auto Gamma counter with a counting efficiency of 85% for ^{125}I. Each point in the assays was determined in triplicate. Statistical significance was determined by the Student *t* test.

Results

Temperature dependence studies revealed that after 3 hours of incubation, at 4° C, 22° C, and 37° C, maximal binding occurred at 4° C, as shown in Fig. 1.

To determine the optimal time for triiodothyronine binding, the samples were incubated at 4° C for 3, 6, 20, and 24 hours. It was found that incubation of the samples for 20 hours yielded maximal binding, as seen in Fig. 2.

Granulosa cell nuclei from small (1 to 2 mm), medium (3 to 5 mm), and large (6 to 12 mm) follicles were incubated for 20 hours at 4° C to determine which population of granulosa cells would show maximal binding. There was no statistical significant difference in the binding of ^{125}I-triiodothyronine to granulosa cells obtained from small and medium follicles. However, granulosa cells from small follicles bound significantly higher amount of ^{125}I-triiodothyronine compared to granulosa cells from large follicles (Fig. 3).

Tissue dependence of triiodothyronine binding was

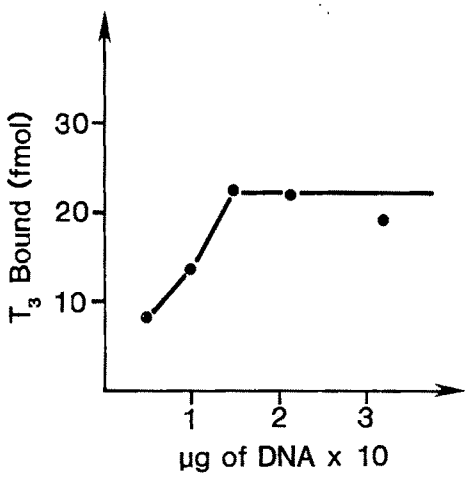


Fig. 4. Deoxyribonucleic acid dependence of triiodothyronine (T_3) binding. Nuclei were isolated from granulosa cells of small follicles (1 to 2 mm). Each assay (in triplicate) was incubated for 20 hours at 4° C.

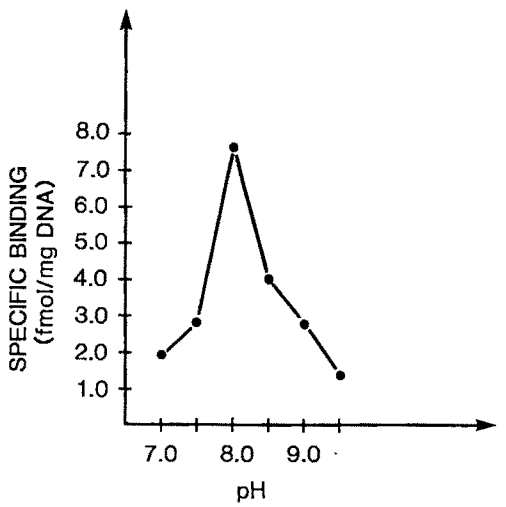


Fig. 5. ^{125}I-triiodothyronine binding as a function of pH. Nuclei were isolated from granulosa cells obtained from small (1 to 2 mm) follicles as described. Each sample (in triplicate) contained 14 to 15 µg of deoxyribonucleic acid and was incubated for 20 hours at 4° C.

Table I. Specificity of ^{125}I-triiodothyronine binding to porcine granulosa cells obtained from small follicles

Hormone	*Inhibition of binding (%)*
Triiodothyronine	50
Thyroxine	8
Reverse triiodothyronine	2
Follicle-stimulating hormone, luteinizing hormone	0
Prolactin	0
Prostaglandins E_2, $F_{2\alpha}$	0
Insulin	0
Epidermal growth factor	0

The concentrations of the different hormones are equivalent to the concentrations of triiodothyronine that caused 50% inhibition of binding (5.0 nmol/L).

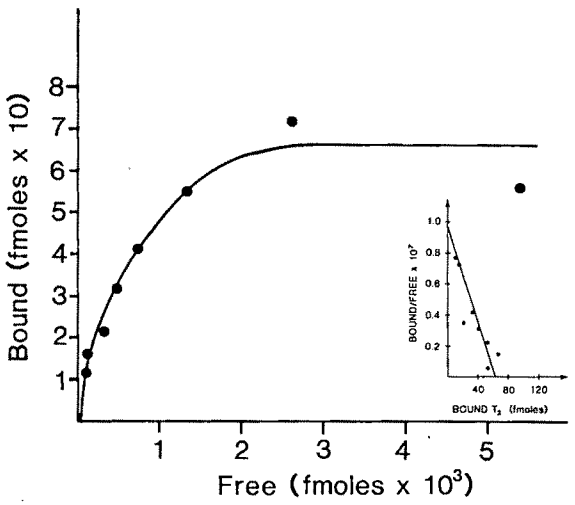


Fig. 6. Representative saturation curve of ^{125}I-triiodothyronine specific binding to nuclei of granulosa cells obtained from small follicles. Each sample (in triplicate) contained 14 to 15 µg of deoxyribonucleic acid, incubated at 4° C for 20 hours at pH 8. *Abscissa* represents free hormone and ordinate bound hormone. The *insert* illustrates a Scatchard plot of the same data.

determined over a range of deoxyribonucleic acid concentrations. As shown in Fig. 4, maximal binding was achieved at 14.44 µg.

The pH dependence of ^{125}I-T3 binding to nuclei of granulosa cells is shown in Fig. 5. The optimal pH for binding was 8.

As a result of the above findings, all further studies were done with nuclei obtained from granulosa cells of small follicles at a concentration of 14 to 15 µg of deoxyribonucleic acid. Incubations were done at 4° C for 20 hours at pH 8.

A representative saturation curve is shown in Fig. 6. This illustrates that the system is saturated under the conditions of the experiment. The Scatchard plot of ^{125}I-triiodothyronine specific binding of the same data shown in the insert of Fig. 6 is linear, indicating the presence of a single set of binding sites. The mean dissociation constant (K_d) for the binding sites obtained from several experiments was 5.5 ± 1.6 (±SEM) nmol with a range of 2.3 to 7.8 nmol. The mean number of triiodothyronine binding sites obtained from several experiments was 1083 ± 402.2 (±SEM) fmol/mg of deoxyribonucleic acid, with a range of 540 to 1867 fmol/mg.

Table I shows the results of specificity studies. Incubation with epidermal growth factor, human prolac-

tin, follicle-stimulating hormone, luteinizing hormone, insulin, and prostaglandins E_2 and $F_{2\alpha}$ in concentrations up to a 200- to 300-fold excess of unlabeled triiodothyronine showed no decrease in binding, whereas incubation with unlabeled thyroxine and reverse triiodothyronine at concentrations equivalent to the unlabeled triiodothyronine concentrations required to inhibit the specific binding of labeled triiodothyronine by 50% (about 5.0 nmol) showed only 8% and 2% inhibition of binding, respectively.

Comment

These studies demonstrate for the first time the presence of triiodothyronine binding sites in porcine granulosa cells. The affinity of these sites ($K_d = 5.5 \pm 1.6 \times 10^{-9}$ mol/L) and the number (1083 ± 402.2 fmol/mg of deoxyribonucleic acid) are comparable to those observed for triiodothyronine receptors in other mammalian tissues.[6-12] Our finding that triiodothyronine binding to porcine granulosa cells was maximal at pH 8 is consistent with the findings of others characterizing triiodothyronine receptors in other tissues.[10-11] The affinity of the binding sites in porcine granulosa cells to thyroxine (one-sixth of triiodothyronine affinity) is similar to the affinity of triiodothyronine receptors to thyroxine in other tissues.[13-15] This may reflect the relative contributions of triiodothyronine and thyroxine to hormonal activity.

We have shown that ^{125}I-triiodothyronine binding to granulosa cells from small and medium follicles was similar, while the triiodothyronine binding to granulosa cells from large follicles was significantly lower ($p < 0.01$) compared to those from small follicles. This suggests that triiodothyronine exerts maximal biologic effects early in the follicular development. By the time the follicle is in the preovulatory phase the number of triiodothyronine receptors in the granulosa cells is diminished, possibly by a down-regulating mechanism that is yet to be elucidated.

Thyroid disorders have been associated with a variety of reproductive abnormalities ranging from menstrual disorders such as amenorrhea to infertility and frequent abortions. In long-standing postpubertal hypothyroidism the ovaries may be atrophied. If hypothyroidism occurred before puberty, degenerative changes in the gonads may be found on histologic examination.[13] Alterations in the metabolism of sex hormones and a decrease in the secretion of luteinizing hormone have been offered as explanations for these aberrations in reproductive physiology.[14-15] However, the physiologic significance of these alterations remains uncertain.[13] One interesting hypothesis resulting from our work is that in cases of thyroid disease, female reproductive physiology may be influenced by a direct effect of thyroid hormones on the granulosa cells. An increase or a decrease in the amount of thyroid hormones may affect granulosa cell luteinization and thus changing progestin production by the putative corpus luteum. This may lead to reproductive failure and menstrual disturbances.

In conclusion, we have demonstrated that porcine granulosa cells contain triiodothyronine binding sites. We have shown that granulosa cells obtained from small follicles have more of these binding sites relative to those cells obtained from large follicles. Although the biologic significance of these putative receptors is not elucidated in our experiments, the previous data suggest a role for these sites in the pathophysiologic changes that are seen in cases of thyroid disease.

REFERENCES

1. Kirkland JL, Mukku VR, Hardy M, et al. Evidence for triiodothyronine receptors in human endometrium and myometrium. AM J OBSTET GYNECOL 1983;146:380.
2. May JV, Schomberg DW. Granulosa cell differentiation in vitro: effect of insulin on growth and functional integrity. Biol Reprod 1981;25:421.
3. Barbieri RL, Makris A, Ryan KJ. Effects of insulin on steriodogenesis in cultured porcine ovarian theca. Fertil Steril 1983;40:237.
4. Channing CP, Tsai V, Sachs D. Role of insulin thyroxine, and cortisol in luteinization of porcine granulosa cells grown in chemically defined media. Biol Reprod 1976; 15:235.
5. Richards GM. Modifications of the diphenylamine reaction giving increased sensitivity and simplicity in the estimation of DNA. Anal Biochem 1974;57:369.
6. Eberhardt NL, Valcana T, Timiras PS. Triiodothyronine nuclear receptors: an in vitro comparison of the binding of triiodothyronine nucei adult rat liver, cerebral hemisphere and anterior pituitary. Endocrinology 1978;102: 556.
7. DeGroot LJ, Torpesani J. Triiodothyronine binding to isolated liver cell nuclei. Endocrinology 1975;96:357.
8. Oppenheimar JH, Schwartz HL, Surkis MI. Tissue differences in the concentration soft triiodothyronine nuclear binding sites in the rat liver, kidney, pituitary, heart, brain, spleen, and testis. Endocrinology 1974;95:897.
9. Oppenheimar JH, Schwartz HL, Surkis MI, et al. Nuclear receptors and initiation of thyroid hormone action. Recent Prog Horm Res 1976;32:529.
10. Samuels HH. In vitro studies on thyroid hormone receptors. In: Birnbaumer L, O'Malley RW, eds. Receptors and hormone action. vol 3. New York: Academic Press, 1978;35.
11. Eberhardt NL, Apriletti JW, Baxtar JD. The molecular biology of thyroid hormone action. In: Litwick G, ed. Biochemical actions of hormones. vol 3. New York: Academic Press, 1980;333.
12. Lindenberg JA, Brehier A, Ballard DL. Triiodothyronine nuclear binding in fetal and adult lung and cultured lung cells. Endocrinology 1978;103:1728.
13. Ingbar SH. The thyroid gland. In Wilson JD, Foster DW, eds. Williams' textbook of endocrinology. 7th ed. Philadelphia: WB Saunders, 1985;682.
14. Gordon CG, Southern AL. Thyroid-hormone effects on steroid hormone metabolism. Bull New York Acad Med 1977;53:241.
15. Distiller LA, Sagel J, Morley JE. Assessment of pituitary gonadotropin reserve using luteinizing hormone-releasing hormone (LPH) in states of altered thyroid function. J Clin Endocrinol Metab 1975;40:512.

Epidermal growth factor deficiency during pregnancy causes abortion in mice

Osamu Tsutsumi, M.D., and Takami Oka, Ph.D.
Bethesda, Maryland

The concentration of epidermal growth factor in the submandibular gland and plasma of female mice increased substantially during pregnancy. Pregestational sialoadenectomy (removal of the submandibular glands) attenuated the rise in plasma epidermal growth factor during gestation and reduced the number of mice completing a term pregnancy by approximately 50%. Epidermal growth factor replacement therapy given to these mice during pregnancy improved the outcome of complete pregnancy to 86%. The administration of anti–epidermal growth factor antiserum in a daily dose of 50 or 100 μl to sialoadenectomized pregnant mice increased the percentages of abortion to 67 and 100, respectively. These results suggest that epidermal growth factor may be necessary for the normal course of pregnancy. (AM J OBSTET GYNECOL 1987;156:241-4.)

Key words: Epidermal growth factor, pregnancy, abortion, submandibular gland

The polypeptide epidermal growth factor (molecular weight = 6045) is produced in large amounts in mouse submandibular glands and is also present in the circulation.[1] Hormones such as testosterone and progestins increase the production of epidermal growth factor in the submandibular gland.[2,3] Epidermal growth factor has been shown to stimulate cell proliferation in various types of mammalian cells in culture, but its physiologic function has not been well defined.[1] The concentration of epidermal growth factor in the submandibular gland and plasma has been found to increase during gestation.[4] In this study we examined the possible physiologic role of epidermal growth factor in pregnancy by assessing the effect of epidermal growth factor deficiency on the outcome of pregnancy. Epidermal growth factor deficiency was produced by either removal of the submandibular gland before gestation or administering anti–epidermal growth factor antiserum to pregnant mice. Epidermal growth factor deficiency during pregnancy was found to increase the incidence of abortion, which suggests that epidermal growth factor is necessary for the normal course of pregnancy.

Material and methods

C3H/HeN virgin female mice were obtained from the Small Animal Section of the National Institutes of Health. At 5 to 6 weeks of age, sialoadenectomy (surgical removal of submandibular glands) was performed with the mice under ether anesthesia. Three weeks after the operation, when they were fully recovered, they were mated with mature C3H/HeN male mice of established fertility. Age- and weight-matched female mice with sham operation were also mated at the same time. The presence of a vaginal plug was checked daily in the morning, and the day of its detection was designated as day 1 of pregnancy. Pregnant mice were caged individually and maintained under controlled temperature (25° C) with 12 hours of light (8:00 AM to 8:00 PM) and 12 hours of darkness. Pregnancy was defined as complete when the pregnant mice gave birth to live pups. The number and weight of newborn pups were determined at the time of birth. Mice that did not complete the pregnancy were put to death on day 21 and the uteri were examined with a dissecting microscope. The number of implantation sites, if present, was checked at various times during pregnancy. The crown-rump length of the fetus was measured by a microscope fitted with a graduated eyepiece.

Epidermal growth factor concentrations in the submandibular gland and plasma of virgin and pregnant mice were determined by radioimmunoassay with the use of the liquid phase double-antibody method as reported before.[4] Blood was obtained from the trunk vein with the animal under ether anesthesia and centrifuged. The plasma was aspirated and stored at −20° C until use. Submandibular gland tissues were excised, weighed, and homogenized in phosphate-buffered saline. The homogenate was centrifuged at 12,000 g for 20 minutes at 4° C and the supernatant was removed and stored at −20° C until use.

Antimouse epidermal growth factor antibody pre-

From the Laboratory of Molecular and Cellular Biology, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health.
Received for publication June 12, 1986; revised August 22, 1986; accepted August 27, 1986.
Reprint requests: Dr. Takami Oka, Laboratory of Molecular and Cellular Biology, NIDDK, Building 8, Room 304, National Institutes of Health, Bethesda, MD 20892.

Table I. Epidermal growth factor concentrations in the submandibular gland and plasma of mice during pregnancy

Mice	n	Epidermal growth factor concentrations		
		Submandibular gland (ng/mg wet tissue)	Plasma (ng/ml)	Mice with detectable levels (>0.1 ng/ml) (%)
Normal virgin	8	43 ± 13	0.16 ± 0.07	38
Pregnant, day 1	7	52 ± 20	0.19 ± 0.12	57
Pregnant, day 3	7	64 ± 13	0.31 ± 0.32	57
Pregnant, day 5	7	73 ± 12	0.25 ± 0.22	57
Pregnant, day 7	7	105 ± 30	0.23 ± 0.13	71
Pregnant, day 9	7	113 ± 30	0.26 ± 0.12	86
Pregnant, day 11	6	164 ± 36	0.52 ± 0.58	100
Pregnant, day 15	6	195 ± 35	0.76 ± 0.63	100
Pregnant, day 19	6	197 ± 29	0.67 ± 0.77	100
Sialoadenectomized virgin	7	None	<0.1	0
Pregnant, day 7	7	None	<0.1	0
Pregnant, day 11	4	None	<0.1	0

Results are given as mean ± SD.

pared in rabbit (Collaborative Research, Waltham, Massachusetts) was administered subcutaneously to sialoadenectomized mice and normal controls in a volume of 50 to 100 μl daily between days 5 and 10 of pregnancy. The anti–epidermal growth factor antiserum was capable of binding 200 μg of epidermal growth factor per 100 μl as determined by a modified Ouchterlony[5] double immunodiffusion method. Normal rabbit serum was injected in the same way. Epidermal growth factor (Collaborative Research, culture grade), 5 μg dissolved in 100 μl of physiologic saline, was administered subcutaneously to sialoadenectomized mice daily from day 7 to 19 of pregnancy. The dose and timing of anti–epidermal growth factor antibody and epidermal growth factor injections yielding optimal effects were determined by preliminary experiments. The mice weighed 20 to 25 gm at the time of treatment.

Results

The concentration of epidermal growth factor in the submandibular gland and plasma increased progressively during pregnancy (Table I). In virgin mice 38% had detectable levels of plasma epidermal growth factor but the incidence increased to 100% on day 11 of pregnancy and thereafter. The plasma epidermal growth factor levels of the sialoadenectomized virgin and pregnant mice were below the level of sensitivity of the assay (<0.1 ng/ml). These results suggested that the submandibular gland may be the major source of plasma epidermal growth factor.

All of the normal mice completed 20 days of pregnancy and gave birth to an average of 7.2 ± 1.6 (SD) pups per mother (Table II). The average weight of the pups was 1.35 ± 0.13 (SD) gm at birth. In contrast, 52% of the sialoadenectomized, pregnant mice completed term pregnancy; the average number of pups decreased to 4.2 ± 1.5 (SD) per mother, and the average birth weight was 1.24 ± 0.09 (SD) gm. These values were significantly lower than those of pups born to normal mice ($p < 0.01$). However, the pups born to sialoadenectomized mothers grew normally. Examination of the uteri of sialoadenectomized mice who did not complete term pregnancy revealed that the resorption sites were found in only 20%, whereas eight of 10 sialoadenectomized pregnant mice apparently had no resorption sites.

Administration of anti–epidermal growth factor antiserum to sialoadenectomized pregnancy mice further decreased the number of mice completing term pregnancy (Table II). Daily injection of 50 or 100 μl anti–epidermal growth factor antiserum reduced the pregnancy success levels to 33% and 0%, respectively. Administration of normal serum had no adverse effects on the outcome of pregnancy. Furthermore, treatment of normal pregnant mice with 100 μl of anti–epidermal growth factor antiserum did not appreciably affect the outcome of the pregnancies.

Table III shows the fetal size on day 11 of pregnancy. The fetal crown-rump length was 4.12 ± 0.30 (SD) mm in normal pregnant mice and 3.34 ± 0.58 (SD) mm in sialoadenectomized pregnant mice. Anti–epidermal growth factor treatment given to sialoadenectomized pregnant mice further reduced the fetal crown-rump length to 3.10 ± 0.42 (SD) mm.

The uteri of sialoadenectomized mice were found to develop normally up to day 7 of gestation and contained approximately the same number of implanted sites as those of normal mice, 8.2 ± 1.0 and 8.4 ± 1.5, respectively. Thus it appeared that epidermal growth factor deficiency produced by sialoadenectomy affected

Table II. The effect of pregestational sialoadenectomy, epidermal growth factor replacement, and administration of anti–epidermal growth factor antiserum on the outcome of completed pregnancies in mice

Mice	*n*	*Treatment*	*Incidence of complete pregnancy (%)*	*Significance*
Normal	18	None	100	
Sialoadenectomized	21	None	52	$p < 0.001$
Sialoadenectomized	9	Anti–epidermal growth factor, 50 μl*	33	$p < 0.001$
Sialoadenectomized	7	Anti–epidermal growth factor, 100 μl*	0	$p < 0.001$
Normal	9	Anti–epidermal growth factor, 100 μl*	89	NS
Normal	7	Normal rabbit serum, 100 μl*	100	NS
Sialoadenectomized	7	Epidermal growth factor 5 μg†	86	NS

The data were analyzed by χ^2 test and the probability that the differences from normal mice were significant is shown.
*Given daily on day 5 to day 10 of pregnancy per mouse.
†Given daily on day 7 to day 19 of pregnancy per mouse.

Table III. The number and size of fetuses in normal, sialoadenectomized, and anti–epidermal growth factor treated, sialoadenectomized 11-day-pregnant mice

Mice	*No. of fetuses per mother*	*Crown-rump length (mm)*	*Total No. of fetuses*
Normal	7.8 ± 1.5	4.12 ± 0.30	31
Sialoadenectomized	7.3 ± 1.0	3.34 ± 0.58*	29
Sialoadenectomized and anti–epidermal growth factor treated†	7.3 ± 1.3	3.10 ± 0.42*	29

*Significant differences were found between normal and sialoadenectomized mice ($p < 0.05$) or anti–epidermal growth factor treated sialoadenectomized mice ($p < 0.01$).
†Anti–epidermal growth factor treatment was given to sialoadenectomized pregnant mice daily at a dose of 100 μl from day 5 to day 10 of gestation. Data represent mean ± SD. Four pregnant mice were used in each group.

neither the fertilization and implantation of oocytes nor the embryonic development until day 7 of gestation but at some later stage it induced abortion. Therefore, it is conceivable that epidermal growth factor replacement therapy begun on day 7 might reduce the rate of abortion. The data in Table II indicated that 86% of epidermal growth factor–treated, sialoadenectomized mice completed term pregnancies. Epidermal growth factor replacement therapy, however, did not significantly increase the number and weight of pups at birth.

Comment

The concentration in the submandibular glands and plasma of female mice increased during pregnancy, and sialoadenectomy was found to reduce the circulating level of epidermal growth factor to undetectable levels. These results confirm the previous findings and support the view that the submandibular gland is a major source of plasma epidermal growth factor in mice.[4] It is likely that the increase in submandibular gland epidermal growth factor during pregnancy is caused by a rise in the circulating levels of progestins and/or androgens[6] inasmuch as these steroid hormones have been shown to increase the concentration of epidermal growth factor in the gland.[2, 3]

Our study also shows that a significant number of sialoadenectomized pregnant mice were unable to complete a term pregnancy. However, this defect was largely corrected by epidermal growth factor replacement therapy during pregnancy. In addition, administration of anti–epidermal growth factor antiserum to sialoadenectomized pregnant mice decreased the likelihood of completing a term pregnancy. These results suggest that epidermal growth factor is important to the normal course of pregnancy in mice.

Anti–epidermal growth factor antiserum given to sialoadenectomized mice further exacerbated the unfavorable outcome of pregnancies. The plasma levels of epidermal growth factor in these mice were too low to be detectable. These findings raise the possibility that there may be other source(s) of epidermal growth factor beside the submandibular gland. Indeed, the presence of immunoreactive epidermal growth factor–like substance in the uterus has been reported.[7] It is conceivable that the uterus produces such a substance that plays a role in the process of pregnancy.

At present, the mode of involvement of epidermal growth factor in pregnancy is not known. The finding that epidermal growth factor replacement therapy was largely effective when it was started on day 7 of pregnancy, at which time implantation has already taken place,[8] suggests that epidermal growth factor plays a role in the postimplantation period. Our data indicate that deficiency of epidermal growth factor results in reduced development of the fetus and an increased rate of abortion. Possible target organs for epidermal growth factor include the fetus and the uterus, both of which have been shown to possess epidermal growth factor receptors.[9-12] It is also possible that epidermal growth factor helps to maintain pregnancy by facilitating the production of female sex steroid hormones in the ovary.

Previously, epidermal growth factor has been implicated in the growth of the mammary gland, another organ that undergoes extensive development during pregnancy.[13, 14] These studies, together with the present findings, suggest that epidermal growth factor is an important physiologic agent for cellular growth and development during pregnancy.

REFERENCES

1. Cohen S, Savage CR. Recent studies of the chemistry and biology of epidermal growth factor. Recent Prog Horm Res 1974;30:551.
2. Byyny RL, Orth DN, Cohen S, Doyne ES. Epidermal growth factor: effects of androgens and adrenergic agents. Endocrinology 1974;95:776.
3. Bullock LP, Barthe PL, Mowszowicz I, Orth DN, Bardin CW. The effect of progestins and submaxillary gland epidermal growth factor: demonstration of androgenic, synandrogenic and antiandrogenic actions. Endocrinology 1975;97:189.
4. Kurachi H, Oka T. Changes in epidermal growth factor concentrations of submandibular gland, plasma and urine of normal and sialoadenectomized female mice during various reproductive stages. J Endocrinol 1985;106:197.
5. Ouchterlony O. Antigen-antibody reactions in gels. Acta Pathol Microbiol Scand 1949;26:507.
6. Barkley MS, Geschwind II, Brandford GE. The gestational pattern of estradiol, testosterone and progesterone secretion in selected strains of mice. Biol Reprod 1979; 20:733.
7. Gonzalez F, Lakshmanan J, Hoath S, Fisher DA. Effect of oestradiol-17β on uterine epidermal growth factor concentration in immature mice. Acta Endocrinol 1984;105: 425.
8. Bindon BM. Pituitary and ovarian mechanisms in implantation. J Reprod Fertil 1973;18(suppl):167.
9. Mukku VR, Stancel GM. Receptors for epidermal growth factor in the rat uterus. Endocrinology 1985;117:149.
10. Nexø E, Hollenberg MD, Figueroa A, Pratt RM. Detection of epidermal growth factor-urogastrone and its receptor during fetal mouse development. Proc Natl Acad Sci USA 1980;77:2783.
11. Sheets EE, Tsibris JCM, Cook NI, Virgin SD, DeMay RM, Spellacy WN. In vitro binding of insulin and epidermal growth factor to human endometrium and endocervix. Am J Obstet Gynecol 1985;153:60.
12. Chegini N, Rao CV. Epidermal growth factor binding to human amnion, chorion, decidua, and placenta from mid and term pregnancy: quantitative light microscopic autoradiographic studies. J Clin Endocrinol Metab 1985; 61:529.
13. Taketani Y, Oka T. Possible physiological role of epidermal growth factor in the development of the mouse mammary gland during pregnancy. FEBS Lett 1983;152:256.
14. Okamoto S, Oka T. Evidence for physiological function of epidermal growth factor: pregestational sialoadenectomy of mice decreases milk production and increases offspring mortality during lactation period. Proc Natl Acad Sci USA 1984;81:6059.

Intrauterine growth retardation after long-term maternal salicylate administration in the rabbit

Jeffrey C. Lukas, M.D., Ted S. Rosenkrantz, M.D., John R. Raye, M.D., Patricia J. Porte, and Anthony F. Philipps, M.D.

Farmington, Connecticut

The effects of chronic maternal salicylism on fetal growth were studied in chronically catheterized pregnant rabbits. Graded intravenous infusions of sodium salicylate were given continuously between days 22 and 29 of gestation. Maternal plasma salicylate concentrations (mean ± SD) of 12.0 ± 1.6 mg/dl (low-dose group) or 24.1 ± 5.3 mg/dl (high-dose group) were achieved. Control rabbits were infused with saline solution. Pups were delivered by hysterotomy on day 29. Fetal/maternal salicylate concentration ratios were near unity for both infusion groups. There were significant dose-related reductions (mean ± SD) in fetal weight (control, 39.7 ± 6.7 gm; low-dose group, 34.4 ± 6.4 gm; high-dose group, 22.2 ± 7.1 gm; $p < 0.001$) and in crown-rump length (control, 9.7 ± 0.45 cm; low-dose group, 9.1 ± 0.68 cm; high-dose group, 7.7 ± 0.86 cm; $p < 0.001$). There was a significant reduction in fetal brain weight only in the high-dose group, and brain weight/fetal weight ratios were increased, suggesting relative sparing of brain growth. Liver weight was significantly reduced in both low- and high-dose groups. In contrast to results in previous animal studies, standardized intravenous maternal salicylate administration in rabbits induced a reproducible and dose-dependent asymmetrical fetal growth retardation. (Am J Obstet Gynecol 1987; 156:245-9.)

Key words: Salicylates, maternal, fetal, growth, intrauterine growth retardation

The widespread use of aspirin during pregnancy[1] has prompted several investigations of the effects of salicylate ingestion during pregnancy on fetal development. Reduced fetal growth has been reported as one such consequence in several animal studies[2, 3] but was an inconsistent finding in others.[4] Clinical studies in humans regarding the effects of salicylates on fetal growth are conflicting as well. An Australian study reported a significant reduction in birth weight associated with high-dose maternal salicylate ingestion[5, 6] while a large retrospective study in the United States[7] with less use of medication by mothers could find no such relationship. In both animal and clinical studies various parameters describing drug exposure, including route and length of salicylate administration, dosage, and amount absorbed, were inconsistent in some cases and poorly defined in others. Given this variability in both methods and results, the purpose of the present study was to investigate the role of salicylates as a possible retardant of fetal growth in late gestation. A chronically catheterized pregnant rabbit preparation was used with defined sodium salicylate intravenous infusions in the last trimester of gestation to examine the fetal effects of salicylate exposure during this period of rapid fetal growth.

From the Departments of Pediatrics and Obstetrics and Gynecology, The University of Connecticut Health Center.

Supported by grants from the University of Connecticut Research Foundation and the National Institutes of Health (AM26067).

Received for publication June 30, 1986; accepted September 10, 1986.

Reprint requests: Anthony F. Philipps, M.D., University of Connecticut Health Center, Farmington, CT 06032.

Material and methods

Eleven New Zealand White does were studied according to the guidelines for care and use of laboratory animals of the Department of Health and Human Services. At operation, with the animals under light ketamine anesthesia, polyvinyl catheters were implanted into the external jugular vein. The catheters were tunneled subcutaneously to the nape of the neck, leaving approximately 1 cm of catheter exposed. After a recovery period of 7 days, the does were mated and divided into three groups: a low-dose group with target maternal plasma salicylate levels of 10 to 15 mg/100 ml (four does), a high-dose group with target salicylate concentrations of >20 mg/100 ml (three does), and a saline-infused control group (four does). On day 19 of gestation, the rabbits were placed into an infusion harness consisting of a vinyl neck collar attached to a vest attached around the thorax of the animal. An extension of the catheter coursed from the infusion harness through a protective metal spring tether loosely attached to the top of the cage, allowing for free access to food and water. After 3 days' acclimation to the har-

Table I. Maternal and fetal study characteristics

	Control	*Low-dose*	*High-dose*
No. of litters	4	4	3
No. of offspring	31	39	26
Average litter size	7.8	9.8	8.7
No. of fetal deaths	0	0	3
Maternal weight change during pregnancy (kg) (mean ± SD)	−0.012 ± 0.045	−0.129 ± 0.256	−0.369 ± 0.486

Table II. Salicylate concentrations in maternal and fetal plasma

	Low-dose	*High-dose*
Maternal sodium salicylate level (mg/dl)	12.0 ± 1.59 (n = 4)	24.1 ± 5.28 (n = 3)
Fetal sodium salicylate level (mg/dl)	13.7 ± 6.69 (n = 35)	27.6 ± 10.72 (n = 23)
Fetal/maternal concentration ratio	1.02 ± 0.32	0.86 ± 0.28

ness, the does were weighed and preinfusion blood samples were drawn from an ear vein as blanks for plasma salicylate determinations.

Continuous intravenous infusions were conducted from day 22 to day 29, with the use of an external syringe pump (Orion Instruments, Cambridge, Massachusetts). The four control does were infused with normal saline solution at a rate of 1.2 ml/hr. The four low-dose rabbits were infused with 60 mg of sodium salicylate (Torigian Laboratories, Queens Village, New York) per milliliter of sterile water solution at the same rate to attain a target maternal plasma salicylate level of 10 to 15 mg/dl. For the infusions of the three does in the high-dose group, higher concentrations of the infusate (80 to 120 mg/dl) were infused at the same rate to attain a target level of >20 mg/dl. Daily maternal blood samples were drawn to monitor maternal plasma salicylate levels, which also allowed for adjustment of the high-dose infusate concentration to maintain maternal plasma sodium salicylate levels near the target value for that group.

On day 29, each doe was weighed and final maternal blood samples were drawn before the animals were put to death. The fetuses were delivered by hysterotomy and the following measurements were recorded for each fetus: body weight, crown-rump length, brain weight, liver weight, and placental weight. Fetal blood samples were obtained via cardiac puncture for determination of plasma salicylate concentration.

The salicylate assay performed on plasma samples was done by spectrophotometric analysis of the chelation of iron by salicylic acid. All data are presented as the mean ± SD. Statistical significance of the results was determined with the one-way analysis of variance, Student's unpaired t test, and χ^2 analyses. Linear regression analysis was performed by means of the least-squares method.

Results

Table I presents the pertinent maternal and fetal study characteristics. There was a total of 96 fetuses from 11 gestations. The average litter size was not statistically different between groups. Fetal mortality was noted only for the high-dose group; however, when survivability was compared by χ^2 analysis, the groups were not significantly different. Maternal weight decreased slightly during pregnancy in all three groups; the magnitude of this loss was not significantly different between groups.

As demonstrated in Table II, maternal plasma salicylate levels averaged during the infusion period were significantly different for the two experimental groups: 12.0 ± 1.59 mg/dl for the low-dose group and 24.1 ± 5.28 mg/dl for the high-dose group. When fetal plasma salicylate levels were compared with maternal levels obtained at delivery, fetal:maternal ratios close to 1 were attained.

The fetal growth parameters are presented in Table III. Mean fetal weight was significantly decreased for the salicylate-treated groups (34.4 ± 6.4 and 22.2 ± 7.1 gm for low-dose and high-dose groups, respectively), when compared with the mean fetal weight of 39.7 ± 6.7 gm for saline-treated controls. As shown in Fig. 1, when all fetal data were pooled, a negative relationship between fetal plasma salicylate concentration and fetal weight was observed ($r = -0.64$, $p < 0.001$). In addition, fetal crown-rump length was significantly decreased for both experimental groups when compared with controls. A dose-dependent response, similar to that noted for the reduction in fetal weight, was observed (Fig. 2). Calculated ratios of fetal weight per centimeter of crown-rump length (Table III, Fig. 3), however, revealed significant and dose-related decrements below the control values.

Selected fetal organ weights were compared between control and salicylate-treated groups, as shown in Table III. Placental weight was significantly decreased for both experimental groups. The placental weight:fetal weight ratio was unchanged for the low-dose group and

Table III. Fetal growth parameters compared between control and treated groups

	Control (n = 31)	*Low-dose (n = 39)*	*High-dose (n = 26)*
Fetal weight (gm)	39.7 ± 6.7	34.4 ± 6.4 ($p < 0.001$)	22.2 ± 7.1 ($p < 0.001$)
Crown-rump length (cm)	9.7 ± 0.45	9.1 ± 0.68 ($p < 0.001$)	7.7 ± 0.86 ($p < 0.001$)
Weight:length ratio (gm/cm)	4.07 ± 0.54	3.75 ± 0.48 ($p < 0.001$)	2.81 ± 0.64 ($p < 0.001$)
Placental weight (gm)	6.40 ± 1.49	5.34 ± 1.41 ($p < 0.001$)	4.49 ± 1.03 ($p < 0.001$)
Placental:fetal weight ratio	0.16 ± 0.033	0.16 ± 0.040 (NS)	0.21 ± 0.045 ($p < 0.001$)
Liver weight (gm)	3.03 ± 0.42	2.37 ± 0.73 ($p < 0.001$)	1.50 ± 0.27 ($p < 0.001$)
Liver:fetal weight ratio	0.078 ± 0.012	0.068 ± 0.013 ($p < 0.001$)	0.072 ± 0.018 ($p < 0.05$)
Brain weight (gm)	1.00 ± 0.08	1.04 ± 0.11 (NS)	0.77 ± 0.17 ($p < 0.001$)
Brain:fetal weight ratio	0.026 ± 0.003	0.031 ± 0.005 ($p < 0.001$)	0.037 ± 0.009 ($p < 0.001$)

was significantly increased for the high-dose group compared with that of controls. A striking reduction in liver weight was apparent, with 22% and 50% decrements observed in low-dose and high-dose groups, respectively. The liver weight:fetal weight ratios for both experimental groups were significantly lower than the control group value. A decrease in brain weight (24% decrement) was noted only in the high-dose group. The fetal brain:body weight ratio increased significantly from a control value of 2.6% ± 0.3% of body weight to 3.1% ± 0.5% and 3.7% ± 0.9% of body weight for low- and high-dose groups, respectively ($p < 0.001$).

Comment

Aspirin (sodium acetylsalicylic acid) is a ubiquitous medication. Several surveys have suggested that it is the most commonly used drug during pregnancy.[1] Experimental animal and clinical human studies addressing the possible effects of maternal aspirin ingestion on fetal development have recently been reviewed.[6, 8] Among the adverse effects reported are an increased incidence of congenital anomalies,[6] reduced fetal growth,[2, 3, 5] increased length of gestation,[6] deleterious effects on the fetal circulation (premature ductus arteriosus closure, pulmonary arterial hypertension),[10] and decreased neonatal hemostasis.[11] Studies of the effects of salicylate use on fetal growth in animals have produced varying results. A significant decrease in fetal body weight after maternal ingestion of 250 mg of aspirin per kilogram per day throughout gestation has been reported in rats.[2] A significant reduction in the birth weight of rabbit pups born to does ingesting 250 mg of aspirin per kilogram per day from day 8 to day 12 of a 32-day gestation has been reported.[3] However, other animal studies have found no significant reduction in litter weight, litter size, or individual fetal weights[4] attributable to aspirin.

Human studies are also inconclusive regarding the effect of salicylate ingestion during pregnancy on fetal growth. An Australian study[5, 6] focused on 64 mothers who ingested large quantities of aspirin through habituated use (two to 12 daily doses) of "headache powders" containing 600 to 700 mg per dose of aspirin or related salicylates. A significant reduction in birth weight was found, and this decrease was found to be related to the number of years the preparation had been taken before pregnancy. A dose-dependent response was also suggested. The U.S. Collaborative Perinatal Project[7] examined the birth weight of infants born to 1515 mothers described as experiencing "heavy" exposure to aspirin during pregnancy (defined as aspirin taken at least 8 days a month for at least 6 months during the pregnancy). No significant decrease in birth weight was found in this group. The authors recognized, however, that the magnitude of ingestion of aspirin was less for study mothers than for the subjects in the Australian study.

Significant problems with data interpretation are apparent when the information available from both animal and human salicylate studies is evaluated. Variability in dosage of drug ingested, inconstancy of blood salicylate concentrations, and variation in length of exposure to salicylates during pregnancy make it difficult to draw firm conclusions from these studies regarding the effects of maternal salicylate exposure on fetal growth. In contrast to previous animal studies, this series of experiments performed in chronically catheterized rabbits was designed to assess changes in fetal growth related to a state of chronic and stable maternal salicylism, as defined by serial measurements of ma-

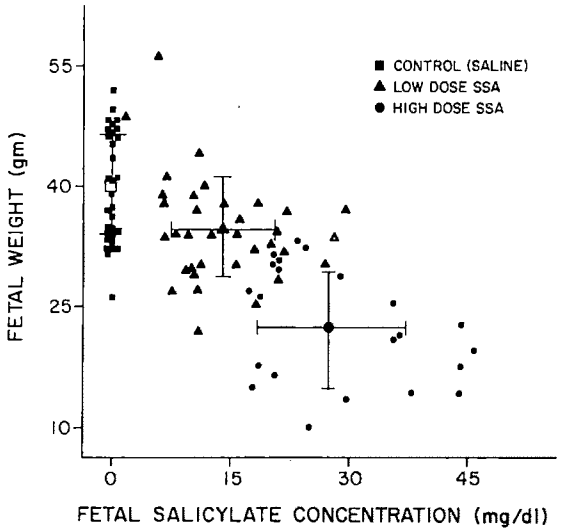


Fig. 1. Relationship between fetal plasma salicylate concentration and fetal weight (fetal weight = −0.51 sodium salicylate, in milligrams per 100 ml, + 40.10, n = 88, r = −0.64, $p < 0.001$).

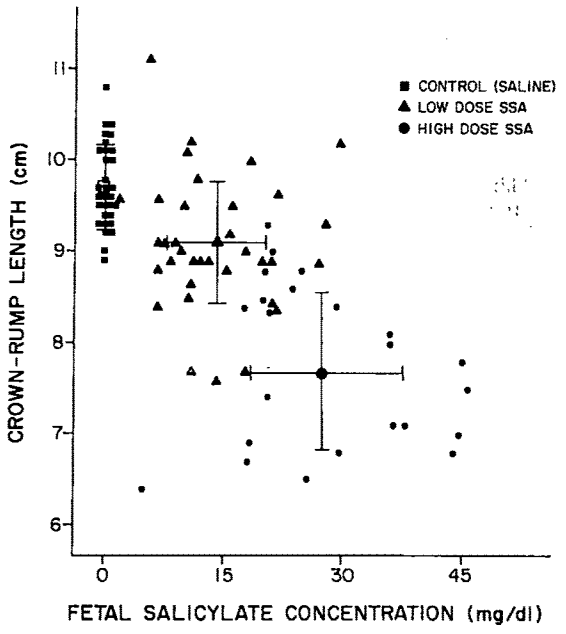


Fig. 2. Relationship between fetal plasma salicylate concentration and fetal crown-rump length (crown-rump length = −0.05 sodium salicylate, in milligrams per 100 ml, + 9.77, n = 88, r = −0.70, $p < 0.001$).

ternal salicylate concentrations in blood. Intravenous administration of salicylates bypassed the inherent variability of oral ingestion and gastrointestinal absorption. Sodium salicylate was used in these series of experiments because of its relative solubility in water when compared with the acetyl ester. Many pharmacologic actions are shared by both drugs, including depression of prostaglandin synthesis via inhibition of the enzyme

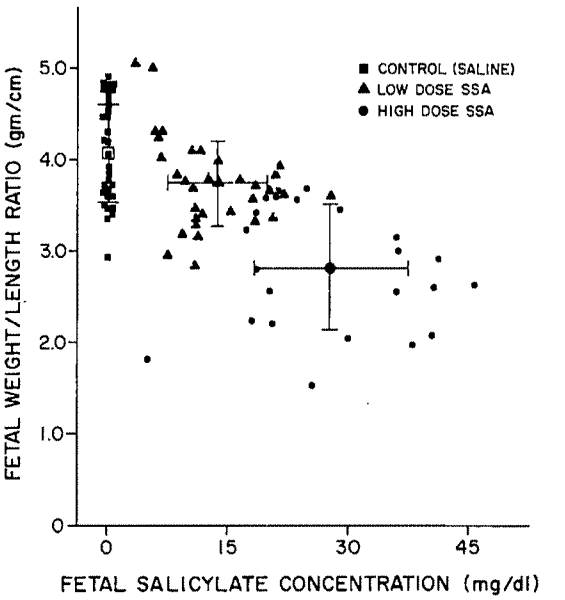


Fig. 3. Relationship between fetal plasma salicylate concentration and fetal weight-length ratio (weight/length = −0.03 sodium salicylate, in milligrams per 100 ml, + 4.06, n = 89. r = −0.60, $p < 0.001$).

cyclo-oxygenase.[1, 12] Most importantly, aspirin is rapidly converted to salicylate after ingestion with 30% to 50% hydrolyzed to salicylate before systemic distribution, both during transport through gut epithelium and during first pass through the liver.[1, 12, 17] In addition, sodium salicylate has little effect on fetal ductal closure[8, 14] and on uterine blood flow,[12, 13] significant causes of fetal mortality in experimental animals[13] in studies with the use of aspirin. The third-trimester infusion period was chosen as a time of most rapid fetal growth.

This series of graded continuous infusions of sodium salicylate in the pregnant rabbit during late gestation resulted in a level of exposure to salicylates in the fetus similar to that in the mother, in agreement with previous observations[1] of fetal: maternal salicylate concentration ratios similar to unity. The present study found a dose-dependent decrement in fetal growth. Fetal weight was affected in greater proportion than skeletal growth as demonstrated by decreased weight: length ratios. There was relative sparing of fetal brain growth with increased brain weight: fetal weight ratios. Liver growth appeared most affected, as shown by significant decreases in liver weight: fetal weight ratios. Placental weights were significantly decreased for both experimental groups, while placental weight: fetal weight ratio was increased only for the high-dose group. This pattern of reduction in fetal body weight and, to a lesser degree, length with relative sparing of brain growth is typical of the asymmetrical intrauterine growth retardation seen in late gestation.

The mechanisms responsible for the inhibition of fetal somatic growth are unclear. Maternal weight gain was poor during pregnancy in this study but no differences between salicylate-treated or saline-infused mothers were observed. It is of interest, however, that previous investigators have noted poor maternal weight gain during salicylate exposure in animals despite adequate food intake.[5] Both aspirin and sodium salicylate are known to be potent inhibitors of aerobic glycolysis and oxidative phosphorylation.[12] Experimental evidence suggests that these effects are not mediated by inhibition of prostaglandin synthesis and that aspirin may be an inhibitor of glycolysis only after conversion to salicylate. Thus a salicylate-induced defect in fuel utilization and/or energy production may have been responsible for the inhibition of both the maternal and the fetal weight gain observed. Alternatively, salicylates have been shown to impair glucose-induced insulin secretion from the fetal pancreas in a dose-related manner.[14] This salicylate-mediated inhibition of insulin release could result in decreased growth, as fetal insulinopenia has been associated with fetal growth retardation in experimental rats[15] and in human case studies.[16]

One can only speculate as to the relevance of this salicylate effect found in rabbits to the human situation. In the human adult, total salicylate concentration 1 hour after ingestion of two standard tablets, or 650 mg, is approximately 7 mg/dl.[17] After ingestion of three tablets, or 975 mg, one could expect a 1-hour postingestion level of at least 10 mg/dl. Thus three aspirins taken every 4 hours could provide peak maternal and fetal plasma salicylate concentrations in the range chosen for the low-dose rabbit infusions in this experiment. This level of ingestion is comparable with that of mothers in the Australian study.

In summary, the present study suggests that chronic sodium salicylate intake in the last trimester of gestation causes a dose-dependent reduction in fetal weight with the development of asymmetrical growth retardation. The mechanisms for the salicylate-induced growth retardation are unclear but may relate to the effects of salicylates on economy of fuel utilization by mother and fetus.

REFERENCES

1. Mandelli M, Morselli PL. Antipyretic and nonsteroid antiinflammatory drugs. In: Morselli PL, ed. Drug disposition during development. New York: Spectrum Publications, 1977:271-309.
2. McColl JD, Globus M, Robinson S. Effect of some therapeutic agents on the developing rat fetus. Toxicol Appl Pharmacol 1965;7:409.
3. Early PA, Hayden J. Effect of acetylsalicylic acid on foetal rabbits. Lancet 1964;1:763.
4. McColl JD, Robinson S, Globus M. Effect of some therapeutic agents on the rabbit fetus. Toxicol Appl Pharmacol 1967;10:244.
5. Turner G, Collins E. Fetal effects of regular salicylate ingestion in pregnancy. Lancet 1975;2:338.
6. Collins E. Maternal and fetal effects of acetaminophen and salicylates in pregnancy. Obstet Gynecol 1981;58:57S.
7. Shapiro S, Monson RR, Kaufman DW, Siskind V, Heinonen OP, Slone D. Perinatal mortality and birth-weight in relation to aspirin taken during pregnancy. Lancet 1976;1:1375.
8. Peterson RG. Consequences associated with nonnarcotic analgesics in the fetus and newborn. Fed Proc 1985; 44:2309.
9. Slone D, Heinonen OP, Kaufman DW, Siskind V, Monson RR, Shapiro S. Aspirin and congenital malformations. Lancet 1976;1:1373.
10. Levin DL. Effect of inhibition of prostaglandin synthesis on fetal development, oxygenation, and the fetal circulation. Semin Perinatol 1980;4:35.
11. Stuart MJ, Gross SJ, Elrad H, Graeber JE. Effects of acetylsalicylic-acid ingestion on maternal and neonatal hemostasis. N Engl J Med 1982;307:909.
12. Packham MA. Mode of action of acetylsalicylic acid. In: Barnett HJM, Hirsh J, Mustard JF, eds. Acetylsalicylic acid: new uses for an old drug. New York: Raven Press, 1982:63-86.
13. Cottle MKW, Van Petten GR, van Muyden P. Effects of phenylephrine and sodium salicylate on maternal and fetal cardiovascular indices and blood oxygenation in sheep. Am J Obstet Gynecol 1982;143:170.
14. Philipps AF, Matty PJ, Porte PJ, Raye JR. Inhibition of glucose-induced insulin secretion by indomethacin and sodium salicylate in the fetal lamb. Am J Obstet Gynecol 1984;148:481.
15. Barash V, Gutman A, Shafrir E. Fetal diabetes in rats and its effect on placental glycogen. Diabetologia 1985;28:244.
16. Lemons JA, Ridenour R, Orsini EN. Congenital absence of the pancreas and intrauterine growth retardation. Pediatrics 1979;64:255.
17. Thiessen JJ. Pharmacokinetics of salicylates. In: Barnett HJM, Hirsh J, Mustard JF, eds. Acetylsalicylic acid: new uses for an old drug. New York: Raven Press, 1982: 49-61.

A monoclonal antibody to a cell surface determinant in human endometrial epithelium: Stage-specific expression in the menstrual cycle

John D. Aplin, Ph.D., and Mourad W. Seif, M.D.
Manchester, England

A monoclonal antibody (CC25) was obtained after immunization of mice with intact glandular epithelial cells from secretory phase endometrium. Here we report a preliminary immunohistologic study in the endometrium of 29 patients in different phases of the menstrual cycle and early pregnancy. In immunofluorescence, CC25 binds to a basolaterally oriented epithelial cell surface antigen that is absent during the proliferative phase and appears suddenly in both glandular and uterine surface locations soon after ovulation. In mid and late secretory phase, the level of expression diminishes slowly. The epitope is absent from glandular epithelial cells in first-trimester decidua. However, it is associated with vascular smooth muscle cells in endometrium and decidua. CC25 promises to be a useful reagent for the analysis of endometrial function during the menstrual cycle. (Am J Obstet Gynecol 1987;156:250-3.)

Key words: Monoclonal antibody, endometrium, cell surface, epithelium, menstrual cycle

Hormone-driven alterations in the structure of the human uterine endometrium during the menstrual cycle are well documented in the histologic literature.[1] Such changes reflect the preparation of the tissue to accept and support an implanting blastocyst 6 or 7 days after ovulation. Relatively little, however, is known about the biochemical changes underlying this remodeling process. Maternal influences over the epithelium, which may occur by hormonal mechanisms or by direct interaction with endometrial stroma, are likely to be exerted at basal epithelial cell surfaces. In contrast, the blastocyst must attach initially to components of apical cell surfaces. Thus functional requirements in the tissue suggest the likelihood of compositional variations between different cell surface domains. The cell surface acts as an important intermediary in the process of preparation for implantation, and so changes in its composition are to be expected corresponding to the different phases of the menstrual cycle.

We have made monoclonal antibodies against cell surface determinants of endometrial epithelial cells and have already demonstrated, using these probes, that the expected asymmetry of composition is present between apically and basally disposed areas of the cell surface[2] (Seif MW, Aplin JD. A monoclonal antibody recognising apical surfaces of glandular but not luminal epithelial cells in human endometrium. Unpublished observations). We now report on an antibody that recognizes an epitope, the expression of which varies dramatically during the menstrual cycle. This marker has considerable diagnostic potential in assessing the response of the target tissue to hormonal changes and ovulation, as well as its preparation to accept the implanting blastocyst.

Material and methods

Endometrial tissue was obtained at curettage in the secretory phase, as judged initially from the last menstrual period and confirmed later by histologic studies. Epithelial cells were isolated and used as immunogen in mice, and antibody-secreting hybridoma cell clones were isolated and recloned as described previously.[2] Screening was by double immunofluorescence on acetone-fixed air-dried cryostat sections of snap-frozen human endometrium from various stages of the menstrual cycle. Scoring (as shown in Fig. 4) was made by comparing intensities of fluorescence of slides stained under identical conditions in which positive and negative control specimens were included in each series. Scoring was on a scale of − to 4+. With practice, scores are reproducible between different observers. Decidua was obtained at termination of pregnancy at 8 to 10 weeks' gestation. It was snap-frozen in liquid nitrogen and sectioned and examined as for endometrium. The epitope recognized by monoclonal antibody CC25 is destroyed by procedures involved in paraffin embedding.

From the Department of Obstetrics and Gynaecology, University of Manchester, St. Mary's Hospital, Manchester M13 OJH, England.
Received for publication March 14, 1986; accepted August 11, 1986.
Reprint requests: John D. Aplin, Ph.D., Department of Obstetrics and Gynecology, University of Manchester, St. Mary's Hospital Manchester, M13 OJH, England.



Fig. 1. Immunofluorescent staining of early secretory phase endometrium with monoclonal antibody CC25. Note fluorescence associated with basolateral surfaces of glandular cells. (×600.)

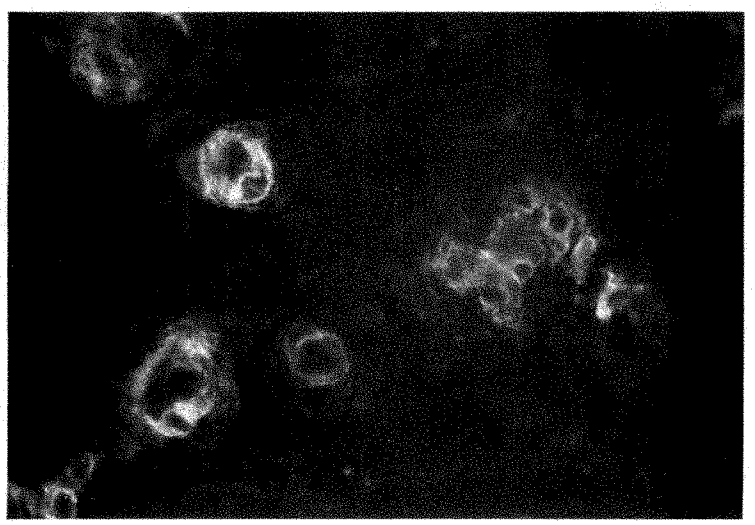


Fig. 2. Immunofluorescence of mid secretory phase endometrium demonstrating the CC25 epitope in the media tunica of small blood vessels. (×600.)

Results

Antibody CC25 recognizes an epitope associated with the basolateral surfaces of epithelial cells both in glandular (Fig. 1) and uterine luminal endometrium. Staining is absent from apical cell surfaces of epithelial cells and the underlying stroma is also negative. Smooth muscle cell layers in the walls of small and medium sized blood vessels are also positive (Figs. 2 and 3).

The CC25 epitope shows a strong menstrual cycle variation (Fig. 4), being completely absent from glandular and luminal epithelial cells in the proliferative phase and making a sudden appearance shortly after ovulation (Figs. 1 and 4).

The exact timing of this appearance in the epithelium in response to ovulation must await further study, since curettings from early secretory phase are obtained infrequently. In mid and late secretory phases, staining with CC25 shows a gradual reduction in intensity (Fig. 4) and a slightly more diffuse distribution based on scoring of different phase endometria stained under the same conditions. The epitope is still confined to basolateral areas of epithelial cells, however, being absent from apical surface. By the time of the menstrual phase, it is lost. In one case of "variable secretory activity" (as assessed by standard histologic examination) the intensity of staining also varies in different glands

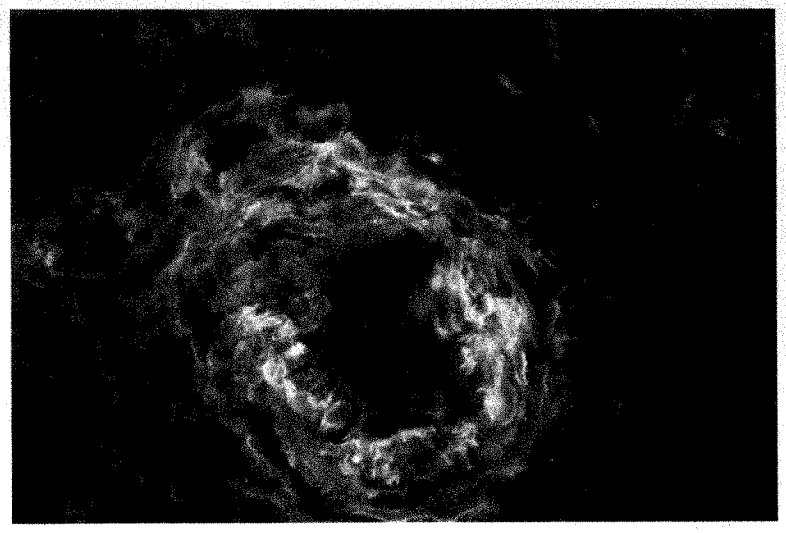


Fig. 3. Immunofluorescence demonstrating the presence of CC25 epitope in a decidual blood vessel. Some interstitial staining is also visible. (×600.)

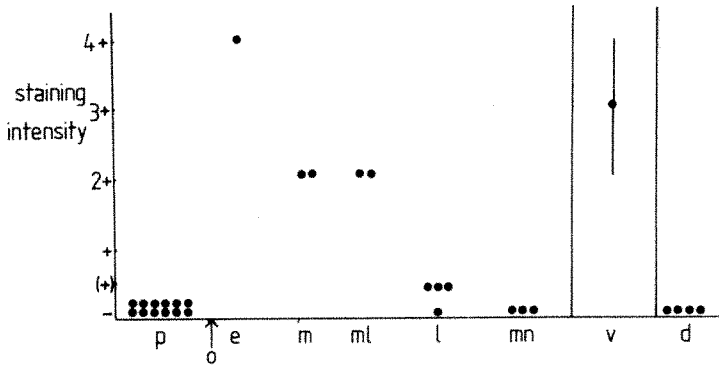


Fig. 4. Expression of CC25 in glandular endometrial epithelial cells assessed by imunofluorescence in 29 patients at different stages of the menstrual cycle and in the first trimester of pregnancy: *p*, proliferative; *o*, ovulation; *e*, early secretory; *m*, mid secretory; *ml*, mid-late secretory; *l*, late secretory; *mn*, menstrual; *v*, variable secretory activity; *d*, first-trimester decidua.

(Fig. 4). This variation (from moderately to strongly positive) is apparent between adjacent glands in the same field of view. Vascular smooth muscle cell layers, in contrast, stain throughout the menstrual cycle with equal intensity (Fig. 2). This staining persists in first-trimester decidua (Fig. 3), where some scattered staining is also associated with a subpopulation of interstitial cells.

Comment

The present study represents an attempt to analyze at a molecular level the changes that occur in the structure of endometrial tissue during the menstrual cycle to provide new parameters for the evaluation of function. The epitope recognized by CC25 undergoes a rapid increase of expression in glandular cells from an undetectable level in proliferative phase to a maximal level after ovulation. The exact timing of this change and the nature of its hormonal control requires further study with use of carefully selected groups of patients in whom hormonal measurements are also available. However, the observation that in a sample of 12 proliferative phase endometria (as assessed clinically and histologically) all were negative suggests strongly that the appearance of the CC25 epitope represents an early response to hormonal changes occasioned by ovulation. Simple dependence on progesterone is ruled out by the absence of the marker from gland cells in the first trimester of pregnancy and by its association with vascular smooth muscle throughout the cycle and in the first trimester. CC25 therefore recognizes a novel marker of endometrial function. The location of the

epitope at basolatleral cell surfaces in glandular as well as surface epithelium suggests a function associated with the response of the epithelial cells to the maternal environment, and biochemical characterization of the carrier molecule may provide insight into its function.

As a marker related to the earlier secretory phase endometrium, however, the CC25 epitope provides a tool for the assessment of endometrial function in a range of clincial situations. These include the detection of ovulation, the staging of the endometrial response, and the diagnosis of abnormal endometrial function. Such approaches may aid in evaluation of patients undergoing artificial insemination and in vitro fertilization, as well as in assessment of subsequent ability of the endometrium to support a pregnancy. They may also add a new parameter to the diagnosis of infertility arising from endometrial dysfunction in response to ovulation.

REFERENCES

1. Dallenbach-Hellweg G. Histopathhology of the endometrium. Berlin: Springer-Verlag, 1981.
2. Aplin JD, Seif MW. Basally located epithelial cell surface component identified by a novel monoclonal antibody technique. Exp Cell Res 1985;160:550.

Comparison of serum progesterone levels in dogs after administration of progesterone by vaginal tablet and vaginal suppositories

L. David Fulper, M.S., Robert W. Cleary, Ph.D., Ernest C. Harland, D.V.M., Ahmed H. Hikal, Ph.D., and Alan B. Jones, Ph.D.
University, Mississippi

Serum progesterone levels from a vaginal tablet formulation and six different vaginal suppository formulations, each containing 25 mg of progesterone, were evaluated in mongrel dogs. Bioavailabilities relative to an intravenous dose of progesterone were calculated. The vaginal tablet was found to have a significantly higher bioavailability compared with the vaginal suppositories. (Am J Obstet Gynecol 1987;156:253-6.)

Key words: Progesterone, suppositories, vaginal tablets

Absorption of progesterone from the intestinal tract is prompt, but the compound is rapidly transformed as a result of the first-pass effect of the liver and possibly also during absorption through the intestinal mucosa.[1] In the 1950s, orally effective compounds with progestational activity (progestins) became available. However, the actions of these compounds differ in some respects from that of progesterone.[2] For this reason progesterone supplementation is often accomplished via vaginal suppository.[3,4]

The vehicle used in the manufacture of a suppository can influence the bioavailability of progesterone.[5,6] Water-soluble polyethylene glycol bases and fatty type bases such as cocoa butter have been used in progesterone suppository formulations. Vaginal tablets have been used to deliver drugs such as nystatin and clotrimazole; however, no progesterone vaginal tablet formulations have been reported in the literature.

To assess the performance of a vaginal tablet formulation of progesterone, the in vivo release of progesterone from a vaginal tablet and six suppository formulations have been compared.

From the School of Pharmacy, Pharmaceutics Department, University of Mississippi.
Received for publication May 19, 1986; revised July 21, 1986; accepted August 14, 1986.
Reprint requests: David Fulper, Pharmaceutics Department, University of Mississippi, University, MS 38677.

Material and methods

Progesterone vaginal tablets containing 25 mg of progesterone were prepared by wet granulation on a Korsch single-punch tablet press (Eweka-G.m.b.H., Frankfurt, Germany) with the use of a 15 mm double concave punch and die set. The tablet weights were 1 gm nominal. Tablet hardness was 8 to 10 kp (kilopond) nominal.

Suppositories containing 25 mg of progesterone were prepared by the fusion method.[7] Micronized progesterone (Paddock Laboratories, Inc., Minneapolis, Min-

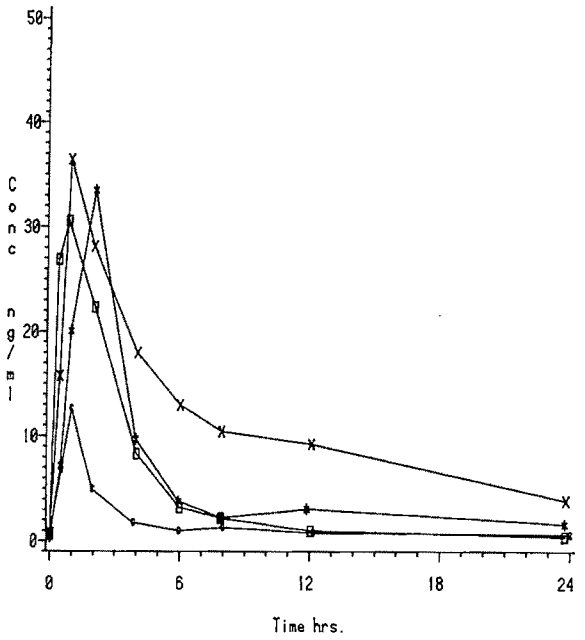


Fig. 1. Serum progesterone levels for vaginal formulations. * = Cetomacrogol 1000; □ = Polybase; X = vaginal tablet; ● = polyethylene glycol.

Table I. Mean area under the curve for progesterone formulations*

Base	*Area under curve (ng · hr/ml)*	*Bioavailability (%)*	*n*
Cocoa butter	15.28 ± 4.70	15.08 ± 4.93	4
Fattibase	18.03 ± 9.60	17.65 ± 9.40	4
Witespol H-15	13.90 ± 6.88	13.64 ± 6.52	4
Cetomacrogol 1000	27.78 ± 6.77	27.02 ± 6.58	4
Polybase	27.50 ± 11.46	26.74 ± 11.15	4
Polyethylene glycol	18.36 ± 8.86	17.89 ± 8.61	4
Vaginal tablet			
Group 1	56.52 ± 5.78	55.82 ± 4.08	4
Group 2	54.31 ± 12.00	52.83 ± 11.67	4
Groups 1 and 2	55.42 ± 8.80	54.30 ± 8.25	8
Intravenous	98.40 ± 9.23	100.00	3

*Complete data available on request.[10]

nesota) was dissolved in the melted base and poured into a mold (Armstrong, Millville, New Jersey). The resulting suppositories were 2.8 cm long and 1.1 cm in diameter. The following bases were used for the manufacture of fatty type suppositories: Witespol H-15 (Riches-Nelson, Inc., Greenwich, Connecticut), Fattibase (Paddock Laboratories, Inc., Minneapolis, Minnesota), and cocoa butter (Woltra, New York, New York). The following bases were used to manufacture water-soluble suppositories: Cetomacrogol 1000 (Pflatz and Bauer, Inc., Waterbury, Connecticut), Polybase (Paddock Laboratories, Inc.) and a polyethylene glycol suppository with a base composition of polyethylene

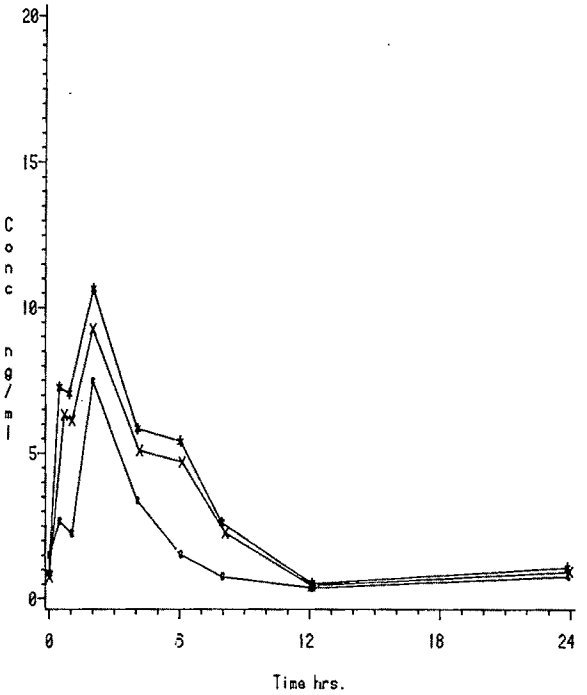


Fig. 2. Serum progesterone levels for vaginal formulations. * = Fattibase; ● = Witespol H-15; X = cocoa butter.

glycol 1500, United States Pharmacopeia, 96% (Fisher Scientific Co., Fair Lawn, New Jersey), and polyethylene glycol 4000, United States Pharmacopeia, 4% (Jefferson Chemical Co. Inc., Houston, Texas).

The water-soluble suppositories and the vaginal tablet formulations were assayed for content uniformity by use of a method reported by Roffe et al.[8] The fatty suppositories were assayed for content uniformity by a modification of the same assay method, whereby the suppository was first dissolved in hexane and an aliquot of this solution was evaporated and reconstituted with methanol before assay.

An intravenous progesterone solution was prepared by dissolving 50 mg of micronized progesterone in 25 ml of ethanol. This solution was diluted 1:1 with normal saline solution immediately before injection.

The vaginal formulations were evaluated in female mongrel dogs weighing between 13 and 23 kg. The ovaries and uterus were removed from each dog approximately 4 weeks before the study and the dogs allowed to acclimate before the study began. The dogs were divided into two groups. Group 1 received fatty-type suppositories and the vaginal tablet. Group 2 received water-soluble suppositories and the vaginal tablet. Dosing order for each group was according to standard Latin square protocol. A 7-day washout period was observed between doses. The dogs were fasted the night before dosing. They were anesthetized with sodium thiamylal and the suppository or tablet was in-

serted vaginally with a plastic tampon applicator. The dogs were allowed to recover from anesthesia and blood was drawn by venipuncture at time 0 and nominally at 0.5, 1, 2, 4, 6, 8, 12, and 24 hours for assay.

Progesterone, 0.25 mg/kg intravenously, was administered to male dogs. The dogs were anesthetized with pentobarbital sodium for the entire experiment. Blood was drawn by venipuncture at time 0 and nominally at 0.08, 0.15, 0.25, 0.50, 1, 2, 4, and 6 hours for assay.

The blood was allowed to clot, and the serum was separated by centrifugation and stored at −40° C, unless the assay was to be performed within 48 hours, in which case it was stored at 10° C. Radioimmunoassay was done with Coat-A-Count progesterone radioimmunoassay kits (Diagnostic Products Corp., Los Angeles, California) according to the manufacturer's protocol.[3] The tubes were counted with an AutoGamma Counter 500C (Packard Instruments, Downers Grove, Illinois).

Results

Because of problems associated with the assay both groups were actually assayed twice. Dog 6 expelled the suppository on week 2 of the second run and died on week 3 of the second run. Inasmuch as the order of dosing was identical in both runs, data from the first run for weeks 2 to 4 were used for this dog in order to maintain a balanced Latin square.

A terminal disposition rate constant for progesterone was calculated from data generated in a separate intravenous study in male dogs. Male dogs were used for the study instead of female dogs mainly for convenience. The data from this study supported preliminary results obtained in female dogs.

Representative plots of the serum data are presented in Figs. 1 and 2. The data for each dog were normalized with respect to dose. The area under the curve from time 0 to infinity was calculated by the trapezoidal rule and the terminal disposition rate constant (obtained from intravenous data). The percent bioavailability was calculated relative to an intravenous dose. The mean and standard deviation for the area under the curve and bioavailability for each formulation are presented in Table I.

Analysis of the combined data from both groups with the use of a one-way analysis of variance resulted in the resolution of two homogeneous subsets of formulations that were statistically different ($p < 0.05$) from each other. Subset 1 contained only suppositories. Subset 2 contained only vaginal tablets. Because there was no statistical difference observed between the vaginal tablets that were common between both groups, the data were pooled and analyzed with a single one-way analysis of variance.

The times to attain peak plasma concentration for the suppositories ranged from 0.6 to 1.3 hours and peak plasma concentration for the vaginal tablet was seen after 1.9 hours.

Comment

Although no statistical difference was demonstrated between the different suppository bases, a clear trend can be seen with respect to bioavailability of progesterone from the water-soluble and fatty type suppositories. It is interesting to note the apparent lower performance of the polyethylene glycol formulation compared with the Polybase formulation, which is also a blend of polyethylene glycols.

The decline in serum concentration of progesterone with the vaginal tablet was slower than that for the suppositories. This behavior is typical of absorption rate–limited elimination. With absorption rate–limited elimination the rate constant for absorption is smaller than the rate constant for elimination, and the time course of the drug in the body becomes a reflection of its absorption from the dosage form. Absorption rate–limited elimination would be expected for drugs with a short half-life such as progesterone.

Assuming concentration-independent kinetics, if two formulations of equal dose are administered by the same route and different areas under the curve are noted, the formulation with the lesser bioavailability is being eliminated before complete absorption can take place. In the case of vaginal administration this is most likely by vaginal expulsion of the formulation. With the exception of dog 6, as noted, no intact suppositories were found in the pens. However, vaginal discharges were occasionally noted after insertion of both suppositories and vaginal tablets. It may be possible that as the suppositories disintegrated, either by dissolution or by melting, they were discharged from the vagina. The faster the disintegration, the sooner the formulation could be expelled from the vagina. This explanation gains some basis when one considers that the polyethylene gylcol formulation, a water-soluble formulation, was designed to dissolve in the same time period that it took the fatty type bases to melt, hence its similarity in performance to cocoa butter, Witespol H-15, and Fattibase formulations.[6] The vaginal tablet, on the other hand, would be expected to absorb fluids as it disintegrates and not form a sufficient amount of fluid to induce an excessive discharge. Cetomacrogol and Polybase, being slower dissolving water-soluble bases, tend to fall between the fast-disintegrating suppositories and the vaginal tablet with its extended residence time.

When a drug is being evaluated for the treatment of a condition, the release profiles of the formulation de-

livering this drug should be considered. The evaluation of the formulations in this study indicates a significant advantage in terms of bioavailability for the vaginal tablet (approximately twice the area under the curve of the next highest formulation) over vaginal suppositories. Increasing the ability of a formulation to resist expulsion from the vagina should be considered to increase the residence time in the vagina and hence the bioavailability.

REFERENCES

1. Goodman LS, Gilman A. The pharmacologic basis of therapeutics. 5th ed. New York: Macmillan, 1975:1436-9.
2. Whitehead MI, Townsend PT, Gill DK, Collins WP, Campbell S. Absorption and metabolism of oral progesterone. Br Med J 1980;281:825-7.
3. Nillius SJ, Johansson EDB. Plasma levels of progesterone after vaginal, rectal, or intramuscular administration of progesterone. Am J Obstet Gynecol 1971;110:470-7.
4. Villanueva B, Casper RF, Yen SCC. Intravaginal administration of progesterone: enhanced absorption after estrogen treatment. Fertil Steril 1981;35:433-7.
5. Buur A, Larsen NC, Rassing M, Hermansen K. Bioavailability of progesterone from rectal dosage forms in rabbits. Int J Pharm 1983;16:41-6.
6. Price JH, Ismail H, Gorwill RH, Sarda IR. Effect of the suppository base on progesterone delivery from the vagina. Fertil Steril 1983;39:490-3.
7. Osol A, ed. Remington's pharmaceutical sciences. 15th ed. Easton, Pennsylvania: Mack, 1980:1550-1.
8. Roffe BD, Zimmer RA, Derewicz HJ. Preparation of progesterone suppositories. Am J Hosp Pharm 1977;34: 1344-6.
9. Diagnostic Products Corporation. Coat-A-Count progesterone. 1985 Feb 13.
10. Fulper LD. Evaluation of vaginal formulations of progesterone [Thesis]. University, Mississippi: University of Mississippi, 1985.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1987 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 156 (January-June) and Vol. 157 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

CORRESPONDENCE

Papanicolaou smears in diagnosis of endocervical chlamydial infection

To the Editors:

We wish to support Dr. Shafer and colleagues (Am J Obstet Gynecol 1985;151:765-71) in concluding that Papanicolaou-stained cervical smears have no place in the definitive diagnosis of endocervical chlamydial infection.

In our study, of 45 women who were chlamydia positive by either culture, monoclonal antibody-stained smears (Microtak, Syva UK), or both, only six (13%) had inclusions in Papanicolaou smears suggesting chlamydial infection.[1] Furthermore, such abnormalities were also found in Papanicolaou smears from 10 (13%) of 76 chlamydia-negative patients. The highly specific and sensitive chlamydial monoclonal antibody test[2] was used to support culture, reducing the likelihood of false negative results. Chlamydial inclusions may be disrupted while obtaining endocervical material or preparing specimens for analysis, but epithelial cells with intact cytoplasmic chlamydial inclusions are seen in <5% of specimens by the monoclonal technique.[1] Therefore it is not surprising that inclusions, characteristic of chlamydial infection, are rarely seen in Papanicolaou smears.

G. E. Forster
I. Cookey, M.B.
Department of Genitourinary Medicine
P. I. Richman, M.B.
R. Jha, M.D.
D. Coleman, M.D.
Department of Pathology
St. Mary's Hospital and Medical School
London W2, England
P. E. Munday, M.D.
B. J. Thomas, Ph.D.
D. A. Hawkins
R. T. Evans, M.I.Biol.
D. Taylor-Robinson, M.D.
Division of Sexually Transmitted Diseases
MRC Clinical Research Center
Harrow, Middlesex, England

REFERENCES

1. Forster GE, Cookey I, Munday PE, et al. Investigation into the value of Papanicolaou stained cervical smears for the diagnosis of chlamydial cervical infection. J Clin Pathol 1985;38:399-402.
2. Thomas BJ, Evans RT, Hawkins DA, Taylor-Robinson D. The sensitivity of detecting *Chlamydia trachomatis* elementary bodies in smears by use of a fluorescein-labelled monoclonal antibody compared to that of conventional chlamydial isolation. J Clin Pathol 1984;37:812-6.

Midforceps deliveries

To the Editors:

I am constrained to reply to the article of Dierker et al. (Midforceps delivery: Long-term outcome of infant. Am J Obstet Gynecol 1986;154:764-8). Nowhere, either in this well-written paper or in the discussion that followed (although there were a number of caveats), did I see any mention of the importance of evaluation of pelvic architecture in assessing the potential outcome of the labor and delivery. Over the years there has been ample documentation of the role of subtle variations in the bony pelvis (android and anthropoid) productive not only of disproportion but of secondary soft tissue insufficiency as well. Clinical evaluation of this structure, it seems to me, should play an important part in a resident's training to better prepare him to make a seasoned judgment in those instances in which reasonable progress in labor is not being made.

Alex S. Tulsky, M.D.
111 North Wabash Avenue
Chicago, Illinois 60602

Reply

To the Editors:

I appreciate Dr. Tulsky's comments regarding our article on midforceps delivery. Maternal pelvic architecture plays a significant role in second-stage dystocia along with other factors such as epidural anesthesia. As mentioned in the article, the role of epidural anesthesia was not prospectively evaluated, so the relationship between epidural anesthesia and the need for midforceps delivery could not be adequately evaluated in our study.

In a similar fashion, pelvic architecture—ranging from clinical adequacy of the pelvis to pelvic type (android, platypellic, etc.)—was infrequently described in the delivery note. Consequently the association between pelvic architecture, difficulty of delivery, and neurological sequelae in the infant could not be addressed. A discussion of pelvic architecture and forceps delivery has been well covered by authors such as Pierce and Danforth in Danforth's text on obstetrics and gynecology.

LeRoy J. Dierker, Jr., M.D.
Department of Obstetrics and Gynecology
Cleveland Metropolitan General Hospital
Case Western Reserve University
3395 Scranton Road
Cleveland, Ohio 44109

Preeclampsia

To the Editors:

Sibai et al. (Maternal and perinatal outcome of conservative management of severe preeclampsia in midtrimester. Am J Obstet Gynecol 1985;152:32-7.) quote our paper[1] as demonstrating the adverse fetal outcome of conservatively managed preeclampsia. Nowhere in our paper is it stated that the 24 patients were "treated conservatively with bed rest and anti-hypertensive therapy." In fact, as stated in the paper, six

Table I. Outcome of all pregnancies complicated by sustained proteinuria and hypertension managed by the high-risk team at the John Radcliffe Maternity Hospital in 1984

	Duration of proteinuria (days)				
	1-7	*8-14*	*15-21*	*>22*	*Permanent*
No. of pregnancies	12	10	10	4	6*
Mean maximum antenatal blood pressure (mm Hg)	182 ± 21/112 ± 11	178 ± 11/122 ± 12	178 ± 24/117 ± 11	185 ± 10/123 ± 5	154 ± 31/102 ± 20
Mean maturity at delivery (days)	215 ± 25	231 ± 21	229 ± 25	221 ± 27	263 ± 2
Range	168-254	199-257	197-260	180-238	224-281
Survival (n)	10	10	10	3	6
Percent	83	100	100	75	100
Reasons for delivery†					
Spontaneous labor	1	0	0	0	1
Maternal	3	3	3	0	1
Fetal	3	1	3	1	0
Maternal plus fetal	1	1	0	0	0
Prophylactic	3	5	4	2	4
Intrauterine death	1	0	0	1	0

*All known to have proteinuria before pregnancy.

†A "maternal" or "fetal" reason for delivery was one where it was considered that the continuation of the pregnancy carried an immediate risk to the safety of mother or child. A "prophylactic" reason was one where it was considered better to deliver than to continue with the pregnancy, but there was no immediate risk to mother or child.

women were delivered within 24 hours of admission and four of these were within 6 hours of admission. Fetal loss occurred in only four cases. Two cases of intrauterine death occurred before the women's admission to the hospital. One case of neonatal death occurred in a woman delivered within hours of admission on fetal and maternal grounds, and one neonatal death occurred at 32 days of age in the case of a woman who had been admitted and treated with Labetalol for 3 days before delivery that was precipitated by a deteriorating nonstress test.

It is our policy to undertake conservative management of proteinuric preeclampsia occurring before 36 weeks only if the following criteria are met: (1) The mother is asymptomatic. (2) Her blood pressure can be controlled at safe levels. (3) There are no biochemical or hematologic abnormalities indicating the pregnancy cannot safely be continued. (4) There is no evidence of fetal distress sufficient to require delivery on nonstress tests, which are usually performed daily.

Failure to apply these criteria will inevitably result in major maternal morbidity as experienced by Sibai et al. The outcome of pregnancies with sustained proteinuria and hypertension managed by the high-risk team at the John Radcliffe Hospital in 1984 is given in Table I. No significant maternal morbidity was experienced.

We would agree with Sibai et al. that much of the maternal morbidity experienced in their study occurred because of delayed delivery, although clearly some of the complications related to inadequate blood pressure control. We believe, however, that in many pregnancies complicated by preeclampsia it is possible to safely undertake conservative management with close maternal and fetal monitoring and immediate delivery when any of the above criteria for conservative management are no longer met.

M. P. Moore, M.B.

Department of Medicine
Princess Margaret Hospital
Christchurch, New Zealand

C. W. G. Redman, M.B.

Nuffield Department of Obstetrics and Gynaecology
John Radcliffe Hospital
Headington, Oxford, England OX3 9DU

REFERENCE

1. Moore MP, Redman CWG. Case-control study of severe pre-eclampsia of early onset. Br Med J 1983;287:580-3.

Reply

To the Editors:

We would like to apologize to both Drs. Moore and Redman regarding the above misquote, which actually applies to another reference. This must have happened during the process of revision and reduction of the number of references in the manuscript. The main reason their study was included in the references is to refer to the four perinatal deaths they reported for gestations ≤29 weeks. This holds true also for other references in our paper.

The rest of the material and data included in their letter is irrelevant to the substance of our report. Their suggestion that some of the complications were related to inadequate blood pressure control is not based on

any solid clinical or scientific data. In fact, there is no evidence to suggest that reducing maternal diastolic blood pressure to <90 mm Hg in these patients will reduce the incidence of any of the complications listed in our report. However, lowering the diastolic blood pressure to <90 mm Hg as suggested by them will increase the incidence of fetal growth retardation, as demonstrated in their report (82% were ≤10 percentile). The patients included in their table are far from being comparable to the patients studied in our report. The definition of preeclampsia used by them applies to mild-to-moderate preeclampsia and not severe (blood pressure ≥140/90 mm Hg or systolic increased by 30 mm Hg and/or diastolic increased by ≥15 mm Hg; 24-hour urine protein ≥1 gm). The patients had severe preeclampsia at time of delivery (as reflected in their maximum antenatal blood pressure values). In addition, the authors excluded all cases of ≤24 weeks' gestation, while 31 patients studied in our report had disease at <25 weeks' gestation.

Moreover, 34 of the 60 patients reported by us received their management elsewhere and then were referred to us because of maternal or fetal complications.

The average gestational age at delivery in our patients was 26.5 ± 1.9 weeks whereas it ranged between 31 ± 3.5 to 33 ± 3.0 weeks in the groups listed in their table. In fact, during a 1-month period in 1984 we encountered a similar number of patients with gestational ages comparable to those listed in that table. Hence the information in that table is irrelevant, since it applies mainly to mild preeclampsia developing during the third trimester.

Baha M. Sibai, M.D.
Division of Maternal/Fetal Medicine
The University of Tennessee, Memphis
853 Jefferson Avenue
Memphis, Tennessee 38163

REFERENCE

1. Moore MP, Redman CWG: Case control study of severe preeclampsia of early onset. Br Med J, 1983, 287:580.

Fetal lung maturity

To the Editors:

I read with great interest the article entitled "Amniotic fluid phosphatidylglycerol and phosphatidylcholine phosphorus as predictors of fetal lung maturity" (Kogon et al. Am J Obstet Gynecol 1986;154:226-30).

The authors state that the analysis of their data was made with the use of standard diagnostic test parameters. However, sensitivity is usually defined as the likelihood that a test will be positive if the disease is present. Conversely, specificity is the likelihood that the test will be normal or negative if the disease is absent.[1]

In this application, the occurrence of respiratory distress syndrome (RDS) is the disease. The positive test result which indicates the possibility of RDS is the absence of phosphatidylglycerol.

Table I. RDS in relation to phosphatidylglycerol determination (<2 days before delivery)

Phosphatidylglycerol	*No RDS*	*RDS*
Present	592 (a)	10 (b)
Absent	28 (c)	36 (d)

Sensitivity $\frac{d}{b+d} = 78.3\%$

Specificity $\frac{a}{a+c} = 95.5\%$

False positive rate $\frac{c}{a+c} = 4.5\%$

False negative rate $\frac{b}{b+d} = 21.7\%$

Positive predictive value $\frac{d}{c+d} = 56.3\%$

Negative predictive value $\frac{a}{a+b} = 98.3\%$

Prevalence $\frac{b+d}{a+b+c+d} = 6.9\%$

In this light, the results of the authors' Table I might be better presented as shown in the Table I of this letter.

William R. Walker, M.D.
Nicolas V. Simon, M.D.
Department of Obstetrics and Gynecology
York Hospital
1001 S. George Street
York, Pennsylvania 17405

REFERENCE

1. Principles of test selection and use: purposes of tests and procedures in the diagnosis of disease. Ann Int Med 1981;94(part 2):559-63.

Reply

To the Editors:

Drs. Walker and Simon make a good point, one which was given great consideration by myself and my coauthors during the writing of the paper.

Sensitivity and specificity are used in the assessment of a test to predict an outcome. This outcome generally is the presence of a disease but not always, as our paper attests.

The presence of the particular phospholipids in amniotic fluid as predictive of an outcome was assessed. That outcome in this case is health (fetal pulmonary maturity) as opposed to disease. In fact, the measure of the outcome was lack of disease (neonatal respiratory distress syndrome). So, although this does not conform exactly to the more usual use of sensitivity and specificity, the format is more appropriate for this study,

inasmuch as the test is to determine a healthful state as opposed to a diseased state.

Drs. Simon and Walker make the point that "the positive test result which indicates the possibility of RDS" is the absence of phosphatidylglycerol. This is not only very different from what we found and wish to state, which is that the positive test result that indicates the improbability of RDS is the presence of phosphatidylglycerol, but it is also very untrue. The results showed that the absence of phosphatidylglycerol is not a good predictor of RDS. That is why the quantification of phosphatidylcholine phosphorus and its correlation to RDS was assessed. In the absence of phosphatidylglycerol, fetal pulmonary maturity could be determined by the measurement of phosphatidylcholine phosphorus.

For this reason it was felt data were best presented as they appeared, but I do thank Drs. Simon and Walker for their time and concern.

David P. Kogon, M.D.

P. O. Box 697
Amherst, Nova Scotia, Canada B4H 4B8

Preterm labor

To the Editors:

In their article (Preterm labor: Its diagnosis and management. AM J OBSTET GYNECOL 1986;154:3-8) Drs. Gonik and Creasy state "no increased iatrogenic risks from preterm serial examinations of the cervix have thus far been identified." Perhaps this depends on the technique of examination (paracervical versus intracervical), because Lenihan[1] showed a significant relationship between antepartum pelvic examinations and premature rupture of the membranes. This may largely explain the failure of Main et al.[2] to reduce premature birth by their interventions; an astounding 50% of the women in their Preterm Labor Prevention Clinic population in Philadelphia had premature rupture of the membranes. Similar pathophysiologic conditions may underlie the threefold increase in premature rupture of membranes caused by recent coitus,[3] and also explain the reported ability of erythromycin to extend gestation in women treated for idiopathic preterm labor.[4] A study looking at the method of cervical examination and recent intercourse in relationship to premature rupture of membranes might be very fruitful.

Jefrey Arlen, M.D.

Mercy Medical Center
1650 Fillmore Street
Denver, Colorado 80206

REFERENCES

1. Lenihan JP. Relationship of antepartum pelvic examinations to premature rupture of the membranes. Obstet Gynecol 1984;83:33-7.
2. Main DM, Gabbe SG, Richardson D, Strong S. Can preterm deliveries be prevented? AM J OBSTET GYNECOL 1985; 151:892-8.
3. Naeye RL. Factors that predispose to premature rupture of the fetal membranes. Obstet Gynecol 1982;60:93-8.
4. McGregor JA, French JI, Reller LB. Adjunctive erythromycin treatment for idiopathic preterm labor. AM J OBSTET GYNECOL 1986;154:98-103.

Reply

To the Editors:

We welcome Dr. Arlen's interest in our article and appreciate the opportunity to respond to his comments. The basis for our statement "no increased iatrogenic risks from preterm serial examinations of the cervix" comes from the personal experiences of one of us with this approach to the high-risk preterm labor candidate. As mentioned in our manuscript, Holbrook et al.[1] could demonstrate no deleterious effect from this approach and was able to identify 18% of high-risk subjects in premature labor by cervical examination alone. In the article by Main et al., which was cited by Dr. Arlen, there were no differences in premature rupture of membranes between those patients serially examined and the control subjects. Additionally, in the article by Lenihan, his data refer to term rather than preterm subjects. We agree with Dr. Arlen that local cervical and vaginal factors may play some role in preterm labor and premature rupture of membranes. However, as pointed out, only a limited amount of data is currently available.

Robert K. Creasy, M.D.
Bernard Gonik, M.D.

The University of Texas
Health Science Center at Houston
Department of Obstetrics, Gynecology and Reproductive Sciences
6431 Fannin, Suite 3.270
Houston, Texas 77030

REFERENCE

1. Holbrook RH, Lirette M, Creasy RK. Weekly cervical examination in the patient at high risk for preterm delivery [Abstract]. Las Vegas, Nevada: Society of Perinatal Obstetricians, 1985.

Ritodrine or terbutaline—The optimal dosage in the treatment of preterm labor

To the Editors:

After reading the report by Beall et al., "A comparison of ritodrine, terbutaline, and magnesium sulfate for the suppression of preterm labor" (AM J OBSTET GYNECOL 1985;153:854-9), we would like to comment on the use of β-sympathomimetic drugs in tocolysis and especially the use of unreasonably high doses of terbutaline. Beall et al. used terbutaline in an infusion rate varying between 20 and 70 μg/min and ritodrine in a maximum dose of 350 μg/min. They present more frequent side effects when using terbutaline compared with ritodrine but otherwise similar results in terms of preventing preterm delivery.

In a recently presented double-blind study comparing ritodrine and terbutaline in the treatment for pre-

term labor, Caritis et al.[1] used maximum terbutaline infusion rates of 17.5 and 350 μg/min of ritodrine. These infusion rates correspond more closely to infusion rates used in northern Europe.

According to Swedish experience, a maximum infusion rate of terbutaline is 25 μg/min[2] although in clinical practice an infusion rate higher than 10 to 15 μg/min is rarely called for.[3]

The maternal side effects of β-mimetics are most likely correlated to the amount of drug administered.

The mechanism of β-adrenoceptor–stimulating agents has been thoroughly studied. Recently we presented a study on the down regulation of β-adrenoceptors in the myometrium after treatment of preterm labor with terbutaline.[4] In a recent study we also measured the concentration of terbutaline in plasma and myometrium from women treated with terbutaline for preterm labor and delivered with cesarean section (Berg G, Rydén G, Andersson RGG, Lindberg C. Down regulation of β-adrenoreceptors in human pregnant myometrium. Unpublished observations). We found an inverse correlation between the concentration of terbutaline in the myometrial tissue and the number of β-adrenoceptors ($r = -0.84$) indicating that the higher the dose administered, the more pronounced the degree of down regulation of the β-adrenoceptor system. Furthermore, as has also been shown by Beall et al., the higher the dosage of β-mimetics, the more frequent and probably the more severe the side effects. Equipotent doses of terbutaline and ritodrine are difficult to state, but an infusion rate of 15 to 20 μg/min of terbutaline probably better corresponds to 350 μg/min of ritodrine than does 70 μg/min of terbutaline.

In conclusion we would like to point out that massive doses of β-mimetics, whether terbutaline or ritodrine, hardly improve the results in treatment of preterm labor but certainly endanger the mother's health. When terbutaline infusion rates >20 μg/min are called for, the mechanisms triggering preterm labor must be seriously looked for and the indication for preventing a preterm birth must be thoroughly scrutinized.

[*Editorial note:* Comments on dosage were given in the reply to Caritis et al. (Beall MH, Paul RH) Am J Obstet Gynecol 1986;155:1357.]

Göran Berg, M.D.
Gunnar Rydén, M.D.
Department of Obstetrics and Gynecology
Linköping University
S-581 85 Linköping, Sweden

REFERENCES

1. Caritis SN, Toig G, Heddinger LA, Ashmead G. A double-blind study comparing ritodrine and terbutaline in the treatment of preterm labor. Am J Obstet Gynecol 1984; 150:7.
2. Ingemarsson I. Effect of terbutaline on preterm labor. A double-blind placebo-controlled study. Am J Obstet Gynecol 1976;125:520.
3. Rydén G. The effect of salbutamol and terbutaline in the management of premature labour. Acta Obstet Gynecol Scand 1977;56:293.
4. Berg G, Andersson RGG, Rydén G. β-Adrenergic receptors in human myometrium during pregnancy: changes in the number of receptors after β-mimetic treatment. Am J Obstet Gynecol 1985;151:392.

Effect of calcium supplementation on the vascular sensitivity to angiotensin II in pregnant women

To the Editors:

Kawasaki et al. (Am J Obstet Gynecol 1985;153: 576-82) have demonstrated that calcium supplementation reduces vascular sensitivity to angiotensin II and that pregnancy-induced hypertension incidence was less (4.5%) in relation to a control group (21.2%), a difference that is statistically nonsignificant.

In the past 2 years we have been involved in a double-blinded study in which supplementation of 2 gm of elemental calcium (gluconate) was given, beginning at the twenty-fourth week of gestation until delivery, to primiparous patients, who were ≤25 years of age and normotensive at the beginning of gestation and who presented no important pathologic alterations. This study was carried out in patients who attended the prenatal clinic in the Obstetrics and Gynecology Hospital Isidro Ayora, Quito, Ecuador. We have demonstrated that pregnancy-induced hypertension, defined in the same terms as those used by the authors mentioned, was present in only 6.5% ($n = 46$) of the calcium-supplemented group. Meanwhile the incidence of pregnancy-induced hypertension in the control group, those who took placebo tablets, was 28.2% ($n = 46$), a difference statistically significant ($p < 0.001$). These results differ from that reported by Kawasaki et al., who in their study only used 156 mg of elemental calcium, in light of the fact that Belizan et al.[1] had suggested earlier the necessity of 2 gm of daily calcium supplementation to maintain constant normal levels of arterial tension during pregnancy.

The high incidence of pregnancy-induced hypertension observed in the control group could be due to known risk factors for these patients, who were primiparous and young (18.6 ± 0.7 years) and had a low daily calcium intake of approximately 300 mg. In the calcium-supplemented group, ionic calcium values, determined by calcium-specific electrode method (Nova II Biomedical, Massachusetts) was maintained constant (1.18 mmol/L ± 0.02) during the pregnancy. In the nonsupplemented group there was an important decrease of the ionic calcium concentration (1.12 ± 0.06 mmol/L) in weeks 28 and 32 of pregnancy.

On the other hand, it has been suggested that prostaglandin I_2 participates in the etiopathogenesis of pregnancy-induced hypertension.[2,3] However, the production of prostaglandin I_2 by the maternal-fetal tissues may be involved in the modulation of the pressor effect of infused angiotensin II.[4,5] We have demonstrated that extracellular calcium concentration directly affects the synthesis of prostaglandin I_2 in vascular tissue while the parathyroid hormone does not, but it can influence the generation of prostaglandin I_2 through changes in ex-

tracellular calcium levels (López-Jaramillo P, Guarner F, Moncada S. Effects of calcium and parathyroid hormone on prostacyclin synthesis by vascular tissue. Life Sci. Unpublished observations). This interesting possibility should be further studied in order to establish the mechanism by which calcium supplementation influences the behavior of blood pressure.

Patricio Lopez-Jaramillo, M.S., M.D.
Marcelo Narvaez, M.D.
Rodrigo Yepez, M.D.
Hospital Gineco-Obstetrico Isidro Ayora
Laboratorio de Investigaciones Facultad de Medicina
Iquique y Sodiro
Quito, Ecuador

REFERENCES

1. Belizan JM, Villar J, Zalazar A, et al. Preliminary evidence of the effect of calcium supplementation on blood pressure in normal pregnant women. AM J OBSTET GYNECOL 1983;146:175.
2. Lewis PJ. Does prostacyclin deficiency play a role in preeclampsia? In: Lewis PJ, Moncada S, O' Grady J, eds. Prostacyclin in pregnancy. New York: Raven Press, 1983:215.
3. Mäkilä UM, Viinikka L, Ylikorkala O. Evidence that prostacyclin deficiency is a specific feature in preeclampsia. AM J OBSTET GYNECOL 1984;148:772.
4. Glance DG, Elder MG, Myatt L. Prostacyclin production and stimulation by angiotensin II in the isolated perfused human placental cotyledon. AM J OBSTET GYNECOL 1985; 151:387.
5. Omini C, Vigani T. Marini A, Pasargiklian R, Fano M, Maselli MA. Angiotensin II: a releaser of PGI_2 from fetal and newborn rabbit lung. Prostaglandins 1983;25:901.

Cesarean sections

To the Editors:

The communique from Boylan and Frankowski (AM J OBSTET GYNECOL 1986;155:455-6), in which they suggested that the frequency of abdominal delivery be also categorized as to parity, inspired me to look closely at the comparable figures generated in our institution.

St. Mary's Hospital in San Francisco is a 550-bed community facility operated by the Sisters of Mercy. The obstetric service is about equally divided between "private" and "clinic" patients, the latter including a high percentage of individuals previously residing in Vietnam, Cambodia, and Laos. Obstetrically, the patients are ones of low- and moderate-risk status, including some who may be considered to be in the high-risk group.

From January 1, 1984, through July 31, 1986, there were 1797 deliveries at St. Mary's. Of these, 340 were delivered by cesarean section, an overall incidence of 19% (the same, in fact, as the overall rate at Hermann Hospital, where Boylan and Frankowski are located).

More pertinent to this missive, 684 of the deliveries were of patients who were para 0. This constitutes 38% of the total. The cesarean section rate in those 684 patients was 23%.

Of the remaining 1113 who were multiparous, the cesarean section rate was 17%.

The fact that there is a difference between rates of abdominal delivery at our hospital and at Hermann Hospital, when viewed by parity, is probably of no great significance. The important reality is that there is a differing section rate between primiparous and multiparous women. I would urge, as do Boylan and Frankowski, that all institutions look more closely at the occurrence of cesarean sections, with regard to the patients' parity.

Now if I may wander a bit, I have long felt that one of the reasons for the alarming increase in cesarean section rates in recent years may be the fact that an ever-expanding proportion of the obstetric population is composed of primiparous women. This fact, in and of itself, would be expected to explain some of the apparent increase in absolute rates of abdominal delivery.

Having said that, I looked back at St. Mary's obstetric population and found to my surprise that 34% of our patients in 1955 were para 0. In other words, the incidence of primiparous women at our hospital is not really much greater now than it was then. (In fact, in 1955 it was slightly higher in proportion than is Hermann Hospital's even now; 32% of all their deliveries in the first half of 1985 constituted primiparous women.)

Presumably, at least at St. Mary's, there is not truly an increasing proportion of primiparous women. That "fact" then is disqualified as a major explanation for the increasing section rate, as I had postulated earlier. (It is interesting but no surprise to those of us who go back a way that the section rate at St. Mary's in 1955 in the case of those primiparous women was only 3%. The overall rate was 4%.)

It would be of interest for other institutions to look at their "ancient" statistics in this manner.

None of these meanderings would, however, mitigate against readers segregating their cesarean section rates as to parity, as did Boylan and Frankowski.

Henry J. Soldati, M.D.
595 Buckingham Way, Suite 500
San Francisco, California 94132

ANNOUNCEMENT

Changes in definitions of clinical staging for carcinoma of the cervix and ovary: International Federation of Gynecology and Obstetrics

The Oncology Committee of the International Federation of Gynecology and Obstetrics at its last meeting (1985) made changes in the definition of some of the stages of carcinoma of the cervix and ovary. These changes are not in the nineteenth annual report on the results of treatment in cases of gynecologic cancer published by FIGO in 1985. Since the twentieth annual report, which will reflect these changes, will not be available until 1988, it was deemed appropriate to make this information available to the readership of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY.

The following are the changes in the definition of clinical staging for carcinoma of the cervix and ovary.

Definition of Stage I carcinoma of the cervix

Stage I. The carcinoma is strictly confined to the cervix (extension to the corpus should be disregarded)

Stage IA. Preclinical carcinomas of the cervix, that is, those diagnosed only by microscopy

Stage IA1. Minimal microscopically evident stromal invasion

Stage IA2. Lesions detected microscopically that can be measured. The upper limit of the measurement should not show a depth of invasion of more than 5 mm taken from the base of the epithelium, either surface or glandular, from which it originates, and a second dimension, the horizontal spread, must not exceed 7 mm. Larger lesions should be staged as IB

Stage IB. Lesions of greater dimensions than Stage IA2 whether seen clinically or not. Preformed space involvement should not alter the staging but should be specifically recorded so as to determine whether it should affect treatment decisions in the future

Notes on staging. Stage IA carcinoma should include minimal microscopically evident stromal invasion as well as small cancerous tumors of measurable size. Stage IA should be subdivided into those lesions with minute foci or invasion visible only microscopically as Stage IA1 and the macroscopically measurable microcarcinomas as Stage IA2 in order to gain further knowledge of the clinical behavior of these lesions. The term IB occult should be omitted.

The diagnosis of both Stage IA1 and IA2 should be based on microscopic examination of removed tissue, preferably a cone, which must include the entire lesion. As noted above, the lower limit of Stage IA2 should be that it can be measured macroscopically (even if dots need to be placed on the slide before measurement) and the upper limit of IA2 is given by measurement of the two largest dimensions in any given section. The depth of invasion should not be more than 5 mm taken from the base of the epithelium, either surface or glandular, from which it originates. The second dimension, the horizontal spread, must not exceed 7 mm. Vascular space involvement, either venous or lymphatic, should not alter the staging but should be specifically recorded as it may affect treatment decisions in the future.

Lesions of greater size should be staged as IB.

As a rule, it is impossible to estimate clinically whether a cancer of the cervix has extended to the corpus. Extension to the corpus should therefore be disregarded.

Stage grouping for primary carcinoma of the ovary

These categories are based on findings at clinical examination and/or surgical exploration. The histologic characteristics are to be considered in the staging, as are results of cytologic testing as far as effusions are concerned. It is desirable that a biopsy be performed on suspicious areas outside the pelvis.

Stage I. Growth limited to the ovaries

Stage IA. Growth limited to one ovary; no ascites. No tumor on the external surface; capsule intact

Stage IB. Growth limited to both ovaries; no ascites. No tumor on the external surfaces; capsules intact

Published with the permission of FIGO.

Stage IC.* Tumor either Stage IA or IB but with tumor on the surface of one or both ovaries; or with capsule ruptured; or with ascites present containing malignant cells or with positive peritoneal washings

Stage II. Growth involving one or both ovaries with pelvic extension

Stage IIA. Extension and/or metastases to the uterus and/or tubes

Stage IIB. Extension to other pelvic tissues

Stage IIC.* Tumor either Stage IIA or IIB but with tumor on the surface of one or both ovaries; or with capsule(s) ruptured; or with ascites present containing malignant cells or with positive peritoneal washings

Stage III. Tumor involving one or both ovaries with peritoneal implants outside the pelvis and/or positive retroperitoneal or inguinal nodes. Superficial liver metastasis equals Stage III. Tumor is limited to the true pelvis but with histologically verified malignant extension to small bowel or omentum

Stage IIIA. Tumor grossly limited to the true pelvis with negative nodes but with histologically confirmed microscopic seeding of abdominal peritoneal surfaces

Stage IIIB. Tumor of one or both ovaries with histologically confirmed implants of abdominal peritoneal surfaces, none exceeding 2 cm in diameter. Nodes negative

Stage IIIC. Abdominal implants >2 cm in diameter and/or positive retroperitoneal or inguinal nodes

Stage IV. Growth involving one or both ovaries with distant metastasis. If pleural effusion is present there must be positive cytologic test results to allot a case to Stage IV. Parenchymal liver metastasis equals Stage IV

*In order to evaluate the impact on prognosis of the different criteria for allotting cases to Stage IC or IIC it would be of value to know if rupture of the capsule was (1) spontaneous or (2) caused by the surgeon and if the source of malignant cells detected was (1) peritoneal washings or (2) ascites.

Corrections

In the article by Hadgu et al., entitled "Predicting acute pelvic inflammatory disease: A multivariate analysis" (Am J Obstet Gynecol 1986;155:954-60), on page 958 under the heading "Prediction outside estimation data," the 500 observations referred to in the second sentence should have read 599 observations.

In the article by Krebs, entitled "Intestinal injury in gynecologic surgery: A ten-year experience" (Am J Obstet Gynecol 1986;155:509-14), in Table II, on page 511, under the heading "Adnexal disease," Total No., given as 1450, should have been 1920; Small bowel %, given as 1.0, should have been 0.7; Large bowel %, given as 0.6, should have been 0.4; Total %, given as 1.5, should have been 1.1.

Roster of American obstetric and gynecologic societies*

(Appears annually in the January issue)

American Board of Obstetrics and Gynecology, Inc. (1930) *President,* Daniel R. Mishell, Jr. *Executive Director,* James A. Merrill, 4507 University Way, N.E., Seattle, WA 98105. Meeting, December 7-11, 1987.

American Gynecological and Obstetrical Society. *President,* Frederick Zuspan. *Secretary,* Henry Thiede, University of Rochester, 601 Elmwood Ave., Rochester, NY 14642. Meeting, September 10-12, 1987.

Central Association of Obstetricians and Gynecologists. (1929) *President,* Stacy R. Stephens. *Secretary,* James H. Maxwell, 320 East Fontanero, Suite 106, Colorado Springs, CO 80907. Meeting, October 15-17, 1987.

Pacific Coast Obstetrical and Gynecological Society. (1931) *President,* Charles F. Langmade. *Secretary,* Walter S. Keifer, 215 Calle Leandro, Cathedral City, CA 92234. Meeting, Sept. 27-Oct. 2, 1987.

Society for Gynecologic Investigation. *President,* Edward Wallach. *Secretary,* Anne Colston Wentz, Vanderbilt University Medical Center, Nashville, TN 37232. Meeting, March 18-21, 1987.

Society of Obstetricians and Gynaecologists of Canada. (1945) *President,* Douglas W. Cudmore. *Secretary,* Patrick T. Mohide, 14 Prince Arthur Ave., Suite 210, Toronto, Ontario, Canada M5R 1A9. Meeting, June 23-27, 1987.

Society of Perinatal Obstetricians. *President,* Amelia Cruz. *Secretary,* Richard Paul, Los Angeles County/University of Southern California Medical Center, Women's Hospital, 1240 N. Mission Road, Los Angeles, CA 90033. Meeting, February 4-7, 1987.

South Atlantic Association of Obstetricians and Gynecologists. (1938) *President,* Leo J. Dunn. *Secretary,* Hobart C. Hortman, Jr., 10 Hospital Circle, Rome, GA 30161. Meeting, January 25-28, 1987.

American College of Obstetricians and Gynecologists. (1951) *President,* Harry S. Jonas. *Secretary,* Ezra C. Davidson, Jr., 600 Maryland Ave., S.W., Suite 300E, Washington, DC 20024-2588. Meeting, April 27-30, 1987.

American Society for Psychosomatic Obstetrics and Gynecology. *President,* Miriam B. Rosenthal. *Secretary,* Dennis H. Smith, University Hospitals, University Circle, Cleveland, OH 44106. Meeting, March 25-29, 1987.

*Changes, omissions, and corrections must be received by the publisher two months in advance (by November 1) for the Roster. Please address the Journal Editing Department, The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146. The number after the society's name is the year of founding. For further information, address the respective secretaries.

Bronx Gynecological and Obstetrical Society. *President,* George J. Kleiner. *Secretary,* Joanna Shulman, 3424 Kossuth Ave., Bronx, NY 10467. Meetings, Jan. 19, March 16, and May 18, 1987.

Brooklyn Gynecological Society, Inc. *President,* Spiro Gallousis. *Secretary,* Frederick H. Sillman, Health Science Center at Brooklyn, 450 Clarkson Ave., Box #24, Brooklyn, NY 11203.

Buffalo Gynecologic and Obstetric Society. (1945) *President,* James J. A. Fraser. *Secretary,* Camille M. Perla, 77 Livingston St., Buffalo, NY 14213. Meetings, Jan. 13, Feb. 10, March 10, April 21, and May 12, 1987.

Chicago Gynecological Society. *President,* Arthur L. Herbst. *Secretary,* Ulisse P. Cucco, 221 W. Pros-

pect Ave., Mount Prospect, IL 60056. Meetings, third Friday of Jan.-June.

Cleveland Society of Obstetricians and Gynecologists. *President,* George Goler. *Secretary,* Gita P. Gidwani, 9500 Euclid Ave., Cleveland, OH 44106. Meetings, Jan. 27, Feb. 18, March 25, and May 27, 1987.

Greater Hartford Obstetrical and Gynecological Society, Inc. *President,* Ashbel G. Gulliver. *Secretary,* David J. Nochimson, University of Connecticut Health Center, OB/GYN, 263 Farmington Ave., Farmington, CT 06032. Meetings, Jan. 3 and April 14, 1987.

Infectious Disease Society for Obstetrics and Gynecology. (1973)

Kansas City Gynecological Society. (1932)

Memphis and Shelby County Obstetrical and Gynecologic Society. *President,* Albert Alexander. *Secretary,* Robert Sauter. Meetings, six times yearly.

Miami Obstetrical and Gynecological Society. (1946) *President,* Chester Morris. *Secretary,* Alex Bezjian, 1150 N.W. 14th St., Suite 214, Miami, FL 33136. Meetings, Thursdays, Jan. 8, March 12, and May 14, 1987.

Milwaukee Gynecological Society. (1951) *President,* William E. Martens. *Secretary,* Mr. Robert Herzog, 850 Elm Grove Rd., Elm Grove, WI 53122. Meetings, Feb. 9, March 9, April 13, Oct. 6, and Nov. 3, 1987.

Montana OB-GYN Society and Montana Section of American College of Obstetricians and Gynecologists. (1948) *President,* William J. Peters. *Secretary,* Thomas C. Olson, P.O. Box 35100, Billings, MT 59107. Meeting, March 5-7, 1987.

Nassau Obstetrical and Gynecological Society, Inc. *President,* Seymour Siegel. *Secretary,* Paul Mazzarella, 179 Community Drive, Great Neck, NY 11021. Meetings, Feb. 8 and April 27, 1987.

New Jersey Obstetrical and Gynecological Society. (1947) *President,* Bernard Pekala. *Secretary,* Gerard Hansen, 2 Princess Rd., Lawrenceville, NJ 08648. Meeting, June 5-6, 1987.

New York Gynecological Society, Inc. *President,* M. Leon Tancer. *Secretary,* Gerson Weiss, 100 Bergen St., Newark, NJ 07103. Meetings, Jan. 28, March 25, and May 27, 1987.

North Dakota Society of Obstetrics and Gynecology. (1938) *President,* Robert Bury. *Secretary,* Thomas Herzog, 737 Broadway, Fargo, ND 58123. Meeting, Sept. 11-12, 1987.

Obstetrical Society of Boston. *President,* Charles F. Eades. *Secretary,* Richard K. Kennedy, 1180 Beacon St., Brookline, MA. Meetings, Jan. 12, Feb. 9, March 16, and April 20, 1987.

Oklahoma City Obstetrical and Gynecological Society. (1940) *President,* Mike Seikel. *Secretary,* Steven D. Jimerson, 1407 N. Porter, Norman, OK 73071. Meetings, Feb. 9, April 13, and May 11, 1987.

Pittsburgh Obstetrical and Gynecological Society. (1935) *President,* Eberhard Mueller-Heubach. *Secretary,* Charles Diez, Ohio Valley Hospital, McKees Rocks, PA 15136. Meetings, first Monday of Oct.-Dec. and Feb.-May.

San Diego Gynecological Society. (1929) *President,* Elizabeth L. Wilder. *Secretary,* William Quesenberry, 15525 Pomorado Road, Poway, CA 92064. Meetings, fourth Tuesday of Sept.-May.

San Francisco Gynecological Society. (1929) *President,* Emmet Lamb. *Secretary,* Gordon Peacock, 3838 California St., San Francisco, CA 94118. Meetings, Jan. 13, Feb. 10, March 10, April 14, and May 12, 1987.

Seattle Gynecological Society. *President,* Douglas Der-Yuen. *Secretary,* Stig Andersen, c/o Helen Rosberg, Executive Secretary, Dept. OB-GYN RH-20, University Hospital, Seattle, WA 98195. Meeting, Sept. 18-19, 1987.

Texas Association of Obstetricians and Gynecologists. (1931) *President,* Bob Shull. *Secretary,* R. H. Wernecke, #403 Medical Park Tower, Austin, TX 78705. Meeting, Feb. 25-27, 1987.

Wisconsin Society of Obstetrics and Gynecology. *President,* Thomas Hofbauer. *Secretary,* William Martens, 10425 W. North Ave., Wauwatosa, WI 53226. Meeting, July 16-18, 1987.

Books received

Abortion and the Private Practice of Medicine. Jonathan B. Imber. 164 pages. New Haven, Connecticut, 1986, Yale University Press. $15.95.

A Classified Bibliography of Controlled Trials in Perinatal Medicine 1940-1984. Prepared by the National Perinatal Epidemiology Unit, Oxford, United Kingdom, with the support of the World Health Organization. New York, 1985, Oxford University Press. $39.95.

Clinical Ethics. Second Edition. Albert R. Jonsen, Mark Siegler, and William J. Winslade. 202 pages. New York, 1986, Macmillan Publishing Company. $22.50.

Clinical Obstetrics: A Public Health Perspective. Edited by Benjamin P. Sachs and David Acker. 308 pages. Littleton, Massachusetts, 1986, PSG Publishing Company, Inc. No price listed.

Clinics in Obstetrics and Gynaecology. New Clinical Issues in In Vitro Fertilization. C. Wood and A. Trounson, Guest Editors. 172 pages. Philadelphia, 1985, W. B. Saunders. $24.95.

Colposcopy. A Scientific and Practical Approach to the Cervix, Vagina and Vulva in Health and Disease. Malcolm Coppleson, Ellis Pixley, and Bevan Reid. 581 pages, illustrated. Springfield, Illinois, 1986, Charles C Thomas, Publisher. $64.75.

The Complete Pregnancy Workbook. Philip D. Sloane, Salli Benedict, and Melanie Mintzer. 268 pages, illustrated. Chapel Hill, North Carolina, 1986, Algonquin Books of Chapel Hill. $13.95 (soft cover).

Infertility, Contraception and Reproductive Endocrinology. Second Edition. Edited by Daniel R. Mishell and Val Davajan. 704 pages, illustrated. Oradell, New Jersey, 1986, Medical Economics Books. $54.95.

Major Ambulatory Surgery. Edited by James E. Davis. 494 pages, illustrated. Baltimore, 1986, Williams & Wilkins. $69.50.

Middle of the Night Baby Book. Nancy Belbas, Julienne Smerlinder, and Mary Kay Stranik. 97 pages, illustrated. Tucson, 1986, The Body Press. $4.95 (soft cover).

Modern Obstetrics in General Practice. Edited by G. N. Marsh. 448 pages. New York, 1986, Oxford University Press. $29.95 (soft cover).

Prepregnancy Care. Edited by Geoffrey Chamberlain and Judith Lumley. 279 pages, illustrated. New York, 1986, John Wiley & Sons. $25.00.

Progress in Obstetrics & Gynaecology. Volume 5. Edited by John Studd. 412 pages, illustrated. New York, 1985, Churchill Livingstone, Inc. $35.00 (soft cover).

Recherches Recentes sur L'Epidemiologie de la Fertilite. J. Henry-Suchet, M. Mintz, and A. Spira. 280 pages, illustrated. Paris, 1986, Masson. 330 F.

Sensory Disorders of the Bladder and Urethra. Edited by N. J. R. George and J. A. Gosling. 181 pages, illustrated. New York, 1986, Springer-Verlag. $39.00.

Standardization and Quantitation of Diagnostic Staining in Cytology. Edited by Mathilde E. Boon and L. P. Kok. 155 pages, illustrated. Leiden, The Netherlands, 1986, Coulomb Press Leyden. $40.00.

Undergraduate Obstetrics and Gynaecology. M. G. R. Hull, D. N. Joyce, and Gillian Turner. 321 pages. Littleton, Massachusetts, 1986, PSG Publishing Company, Inc. $15.00 (soft cover).

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

Silver Anniversary Symposium on Critical Care Medicine, March 23-26, 1987, Caesars Palace Hotel, Las Vegas, Nevada. Presented by the University of Southern California School of Medicine, The University of Health Sciences/The Chicago Medical School, and The Institute of Critical Care Medicine. For information contact: Associate Dean, USC School of Medicine, Postgraduate Division, 1975 Zonal Ave., KAM 307, Los Angeles, CA 90033. Tel.: (213) 224-7051 or (800) 421-6729 (outside California) or (800) 321-1929 (within California).

Obstetrical Anesthesia: 1987, March 19-22, 1987, Hotel Meridien, San Francisco, California. Sponsored by the University of California, San Francisco, Department of Anesthesia. For further information contact: Anesthesia Research Foundation Registration Office, Attention: Mark A. Rosen, M.D., University of California, San Francisco, C-450, San Francisco, CA 94143-0648. Tel.: (415) 476-2273.

Intrauterine Diagnosis and Treatment: A New Frontier, April 2-4, 1987, Hotel Inter-Continental, San Diego, California. AMA/CMA accreditation: up to 19.5; ACOG and American Academy of Pediatrics, pending. Contact: Office of Continuing Medical Education, M-017, UC San Diego School of Medicine, La Jolla, CA 92093. Tel.: (619) 534-3940 or (619) 452-3940.

Current Concepts in Obstetrics and Gynecology Focusing on Endometriosis, March 27-28, 1987, Inn on the Park, Four Riverway, Houston, Texas. Presented by Baylor College of Medicine, Department of Obstetrics and Gynecology. For information contact: Lila K. Lerner, Program Coordinator, The Office of Continuing Education, Baylor College of Medicine, One Baylor Plaza, Houston TX 77030. Tel.: (713) 799-6020.

High-Risk Obstetrics, April 2-4, 1987, Chicago, Illinois. Sponsored by the Cook County Graduate School of Medicine. For additional information contact the Registrar's Office. Toll-free number: Illinois 1 (800) 621-4649, outside Illinois 1 (800) 621-4651.

XIIth Latin American Congress of Obstetrics and Gynecology, October 25-30, 1987, Camino Real Hotel, Guatemala City, Central America. For further information please contact: Dr. Carlos Raúl Juarez, Secretary Organizing Committee, 7a. Ave. 6-53, Zona 4, Edificio El Triangulo, Local 48, 2°nivel, P. O. Box 2967, Guatemala City, Central America.

Annual Scientific Program and Postgraduate Courses, May 6-10, 1987, the Americana Canyon Hotel, Palm Springs, California. Sponsored by the Pacific Coast Fertility Society. For information contact: Pacific Coast Fertility Society, 5820 Wilshire Blvd., #500, Los Angeles, CA 90036. Tel.: (213) 937-5514.

2nd Heidelberg International Symposium on New Aspects in Female Contraception—Update and Trends, under the patronage of the VIII World Congress of Sexology, June 13-16, 1987, Heidelberg, West Germany. President: Prof. Benno Runnebaum, M.D. For further information contact the Organizing Secretariat: Thomas Rabe, M.D., or Ludwig Kiesel, M.D., Universitäts-Frauenklinik, Voßstr. 9, D-6900 Heidelberg, West Germany. Tel.: 62 21/ 56 50 72.

II Panamerican Symposium on Perinatal Medicine, Hotel Miramar, Viña del Mar, Chile, April 8-11, 1987. For further information contact: Organización TRES Ltda., Ms. Marcela Torres, Casilla 16522, Correo 9, Santiago, Chile. Telex: 340436 PB VTR CL Tres Ltda. Tel.: 2319103-2318211.

Contemporary Obstetrics and Neonatology: Partners in Prematurity, February 25-27, 1987, Doubletree Hotel, Orange, California. Sponsored by the University of California, Irvine, College of Medicine, Division of Neonatal/Perinatal Medicine. For further information contact: Mari Jo Craft, RNC, Program Director, University of California, Irvine, Medical Center, 101 City Dr., Route 81, Orange, CA 92668. Tel.: (714) 634-6162.

COMMUNITY HEALTH CENTER
Westchester County, New York

Seeking OB/GYN specialist due to expansion of services of established Community Health Center located 30 miles north of New York City. Board certified or eligible. Beautiful facility, excellent hospital affiliations, good benefits, including malpractice insurance. Full-time or Part-time beginning early 1987. Salaried. Spanish language helpful. Equal Opportunity Employer.

Submit C.V. to

K. Thompson
Administration
6 Furnace Street
Cold Spring, NY 10516

PERINATOLOGIST
Denver, Colorado

Rose Medical Center, Denver, Colorado, is seeking a BE/BC perinatologist to work full-time in a 400-bed private community hospital adjacent to the University of Colorado Health Sciences Center. With 3600 deliveries per year, and thirty-six University residents rotating through the department annually, Rose is the major private teaching affiliate of the University.

Responsibilities would include private consultation, physician, resident, and nursing education, clinical research, and a limited private practice in perinatology. This individual can expect frequent interaction with the Maternal/Fetal Medicine division at the University of Colorado and would be eligible for an appropriate clinical appointment at the University.

A competitive salary guarantee is being offered with excellent fringe benefits including malpractice insurance.

Qualified applicants, including individuals completing fellowship in June, 1987 should forward CV's to:

Robert E. Wall, M.D.
Chairman, Department of OB/GYN
Rose Medical Center
4567 E. Ninth Avenue
Denver, Colorado 80220

REPRODUCTIVE ENDOCRINOLOGIST

Pennsylvania Hospital seeks Board Certified or eligible specialist with fellowship training in reproductive endocrinology and infertility. Responsibilities include participation in IVF program, resident and medical student education and active clinical practice. Appointment is at the Assistant Professor level. Previous experience in academic medicine is desirable.

Pennsylvania Hospital is an affiliate of the University of Pennsylvania and is an Affirmative Action Equal Opportunity Employer.

Interested candidates should send curriculum vitae to:

Esther Eisenberg, M.D.
Department of Obstetrics and Gynecology
Pennsylvania Hospital
Eighth and Spruce Streets
Philadelphia, PA 19107

Subspecialist Positions

615-bed tertiary care facility is seeking several board eligible or board certified subspecialists for the following areas within an expanding Department of Obstetrics and Gynecology.

- **Maternal/Fetal Medicine**
  Two new positions available in our established division of Maternal/Fetal Medicine. Ultrasound experience desirable.
- **Reproductive Endocrinology**
  An exceptional growth opportunity is available for a physician to direct this new division.

These positions offer excellent opportunities for professional development to qualified physicians interested in subspecialty practice, resident training, and research/faculty status in a large university affiliated hospital.

SINAI HOSPITAL OF DETROIT

Please submit your Curriculum Vitae or contact **Dr. David B. Schwartz, Chairman, Department of Obstetrics and Gynecology, Sinai Hospital of Detroit, 6767 W. Outer Drive, Detroit, MI 48235, or call (313) 493-5220.**

POSITIONS AVAILABLE

BOARD ELIGIBLE, BOARD CERTIFIED

OBSTETRICIANS/GYNECOLOGISTS

FOR PERMANENT POSITIONS
IN MID AND SOUTH FLORIDA

SOME DIRECTORSHIP RESPONSIBILITIES
AVAILABLE

CONTACT:
Harvey Gardy, M.D., F.A.C.O.G.
Broward General Medical Center
1600 South Andrews Avenue, Room 319 West
Ft. Lauderdale, FL 33316
305-463-4368

OR:
Steven Scott, M.D., F.A.C.O.G.
P.O. Box 3079
Durham, NC 27705
919-383-0355

SUNLIFE
OB/GYN
SERVICES, INC.®

MATERNAL-FETAL MEDICINE
(Board eligible or Certified)

600 Bed University-affiliated Level III Hospital with 3,000 deliveries/year, 24-hour in-house anesthesiology and neonatology/large NICU, active maternal transfer service, perinatal outreach programs, and fully-approved OB/GYN residency program has immediate opening for a full-time position in perinatology. Duties to include residency teaching, maternal transfer care, and private practice incentive. Competitive guaranteed salary plus incentive. Excellent public school system, housing, and Lake St. Clair in very close proximity.

CALL COLLECT: 313-343-3495
SAINT JOHN HOSPITAL
DEPT. OB/GYN
DETROIT, MI 48236

Full Time
Faculty Position
Maternal-Fetal Medicine

The department of OB/GYN at Temple University Health Science Center is seeking a Board Certified/Board Eligible Perinatologist. Clinical and academic activities are integrated in a department providing tertiary services, including genetic counseling. Newly opened Temple University Hospital houses a modern Labor & Delivery suite and large Neonatal Intensive Care unit. Approved Fellowship program. Candidates should have an active interest in Research, Clinical Practice, and Resident and Medical student education. Salary and academic rank commensurate with experience. Opportunity for qualified person to become Section Chief. Submit C.V. to:

P. Hanjani, M.D., Professor, OB/GYN

Temple University
Health Science Center
3401 N. Broad St.
Philadelphia, PA 19140

TEMPLE UNIVERSITY

An equal opportunity,
affirmative action employer

GYNECOLOGIC ONCOLOGIST

The Allentown Hospital, with the 5th largest obstetrical service in Pennsylvania, is actively seeking a board certified or board eligible gynecologic oncologist for private practice and to provide leadership and medical education in gynecologic oncology.

The Hospital, a component of the HealthEast System, is located in the very desirable Lehigh Valley, in close proximity to all major East Coast cities. It sponsors a fully accredited OB/GYN residency program and has a major medical school affiliation. The Hospital co-sponsors a Comprehensive Community Cancer Center and offers a special interest gynecology group from multi-disciplinary specialties. There are no Board certified or Board eligible gynecologic oncologists in the Lehigh Valley, and the position provides a superb opportunity to establish a subspecialty practice and be integratly involved in a major regional teaching program.

Submit C.V. in complete confidence to:

Sze-ya Yeh, M.D.
Chairman, Department of Obstetrics/Gynecology
The Allentown Hospital
17th & Chew Streets
Allentown, Pennsylvania 18102
(215) 778-2412

OBSTETRICIAN GYNECOLOGIST

Position available with The Permanente Medical Group, Inc., San Jose, California. Sixteen member OB/GYN Department seeking Board Certified/Eligible physicians for immediate openings. Excellent professional environment, competitive salary, outstanding benefits. Located in the San Francisco Bay Area, with superb educational, cultural and recreational activities.

Contact:

Jerome L. Nehemiah, M.D., Chief
Department of OB/GYN
The Permanente Medical Group, Inc.
260 International Circle
San Jose, CA 95119

ASSISTANT PROFESSOR
(tenure earning)

Department of Obstetrics and Gynecology, Division of Maternal and Fetal Medicine. Board Certified or Board Eligible Maternal and Fetal Medicine subspecialist. Large volume indigent and referral obstetrical service with a major 4-year residency program. Primary responsibilities in teaching, service and research. Competitive salary and fringe benefits commensurate with experience.

Deadline for applications:
January 31, 1987

Contact:

Mr. Robert H. McCorkle
Executive Administrator
Department of Ob/Gyn
UAB
University Station
Birmingham, Ala. 35294

An Affirmative Action/Equal Opportunity Employer

OBSTETRICIAN/ GYNECOLOGIST

Board eligible or board certified obstetrician / gynecologist sought to join an established two-physician practice. Excellent opportunity in a progressive, appealing community, one hour south of Atlanta. Competitive salary and benefits. Recreational facilities in area include hunting, fishing, boating, water skiing, golf, tennis, and skeet. Excellent hospital facilities, schools, and housing.

Submit cirriculum vitae and references to:

OB/GYN
P. O. Box 486
LaGrange, GA 30241

ASSISTANT DIRECTOR
OB/GYN SECTION
FAMILY PRACTICE RESIDENCY
UNIVERSITY OF MASSACHUSETTS
LOWELL GENERAL HOSPITAL

The University of Massachusetts and its affiliated obstetrical section in Lowell, Massachusetts is recruiting for a full-time Assistant Director. This section includes a large obstetrical clinic, a smaller GYN component, and training in high risk and normal obstetrics for Family Practice residents and fellows. There is a strong affiliation with the University of Massachusetts Medical School in Worchester, located 45 minutes from Lowell.

Qualified applicants should be board certified/ board eligible in Family Practice or in Obstetrics and Gynecology. Salary and benefits are through the University and will be commensurate with previous training, skills, and level of appointment.

Applicants should submit letters of inquiry and/or CV to:

Stanley Warner, M.D.
Director for Obstetrical Training
Lowell General Hospital
295 Varnum Avenue
Lowell, MA 01845
(617) 937-6396

An equal opportunity employer

CONFIDENTIAL APPLICATIONS
ARE NOW BEING CONSIDERED FOR A

DIRECTOR OF MEDICAL EDUCATION, DEPARTMENT OF OBSTETRICS AND GYNECOLOGY

St. John's Mercy Medical Center, in St. Louis, MO. is accepting applications for an experienced academician/administrator to assume major responsibility for the four-year, fully-accredited residency program in Obstetrics and Gynecology as well as physician and nursing continuing medical education. This position will report to James G. Blythe, M.D., Chairman of the Department of Obstetrics and Gynecology.

Candidates for this position will:

- be board certified by the American Board of Obstetrics and Gynecology;
- have proven abilities in administration and teaching as well as excellent clinical skills;
- practice at an 859 bed facility with an obstetrical service delivering approximately 6,000 babies annually;
- enjoy an attractive compensation and benefit package as well as medical school affiliation and a private practice component.

For additional information, call or write in confidence:

Phil Gilbert, Administration
St. John's Mercy Medical Center
615 South New Ballas Road
St. Louis, MO 63141
314-569-6116

PERSONALIZED LIBRARY CASES

Protect your personal copies of
AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY
in custom made, titled library cases.

Constructed of durable, reinforced binder's board covered in a rich simulated leather, each is designed to hold an entire year's issues. The Journal name is stamped in gold leaf, making the cases attractive additions to any decor, whether on your bookshelf, desk or any location in your home or office.

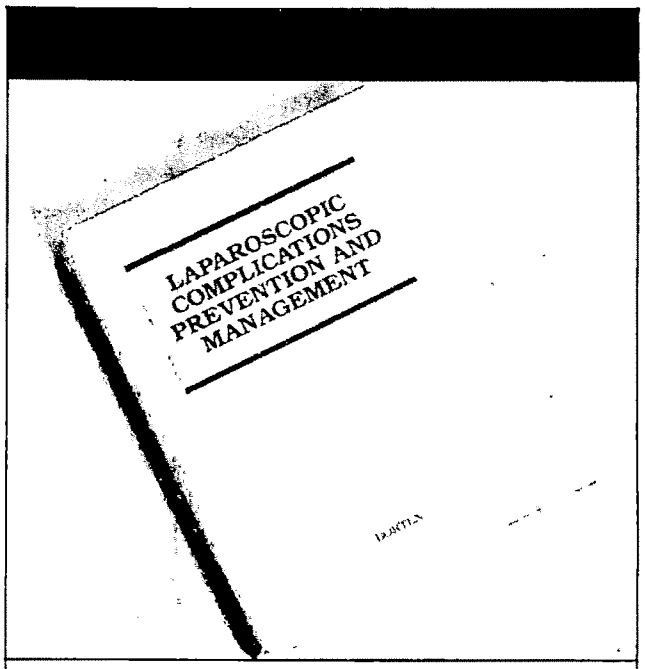
Only $7.95 each: 3 for $21.95; 6 for $39.95, and $1 per unit postage and handling ($2.50 per unit outside the U.S.A.). U.S. funds only.

To charge your order using American Express, Visa, MasterCard or Diners Club, call toll-free: 1-800-972-5858.

Please send me _____ library cases for
AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY
at $7.95 each: 3 for $21.95; 6 for $39.95, and $1 per unit postage and handling ($2.50 per unit outside the U.S.A.).

Name _____
(please print legibly or type)
Address _____
(no P.O. boxes, please)
City/State/Zip _____
PA residents add 6% sales tax

Jesse Jones Industries
499 East Erie Avenue
Philadelphia, PA 19134
Department Code: Mosby



The Latest News in Obstetrics and Gynecology . . .

New!
LAPAROSCOPIC COMPLICATIONS: Prevention and Management

By Max Borten, M.D.
Written by a recognized authority, this book gives you first-hand knowledge on both medical and surgical management of laparoscopic complications. From indications and contraindications, it guides you through detailed discussions of failures of procedure and equipment, to the medicolegal aspects of both the procedure and its complications. Intraoperative slides and line drawings fully illustrate the text. Once you see it, you won't let it go! Order your copy today! 1986. 464 pages. (D-0723-X) Mosby distributes this B.C. Decker, Inc. publication. About $79.50

OUTLINE OF CONTENTS: INTRODUCTION: Instrumentation, Technique, Diagnostic Indications for Laparoscopy, Operative Indications for Laparoscopy, Laparoscopic Sterilization, Contraindications to Laparoscopy, Nongynecologic Indications for Laparoscopy, Choice of Anesthesia, Circulatory Changes, Respiratory Changes, Postoperative Recovery COMPLICATIONS: Equipment Failure, Procedural Failure, Complications of Anesthesia, Complications of Pneumoperitoneum, Complications of Trocar Insertion, Vascular Injuries, Intestinal Tract Injuries, Urinary Tract Injuries, Tegumentary Injuries, Complications due to Infection, Complications of Sterilization, Electrical Complications, Postoperative Complications, Medicolegal Aspects of Laparoscopy APPENDIX: Translaparoscopic Photography

Save time, call FREE 1-800-345-8500, ext. 15. Our hotline is open 24 hours a day, 7 days a week.

AMS-938-031

B.C. Decker MOSBY TIMES MIRROR

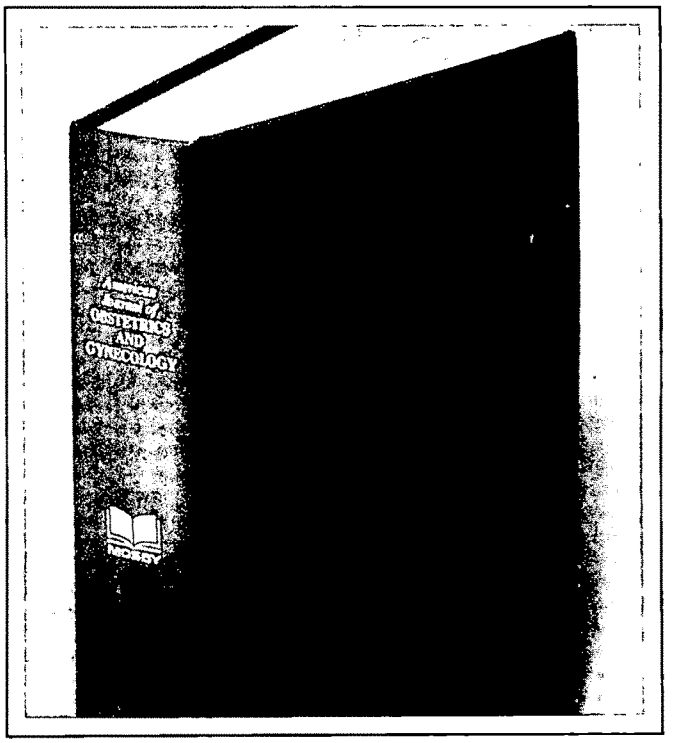
FREE for 30 days! Please send me _____ copies of: Borten, LAPAROSCOPIC COMPLICATIONS: Prevention and Management (D-0723-X) About $79.50

Please print or type.
☐ Bill me, plus shipping charges ☐ Payment enclosed
☐ MasterCard ☐ VISA
Card # _____ Exp. date _____
Name (first, midd e, last) _____
Address _____
City _____ State _____ Zip _____
Bus. phone ___ - ___ - _____ Specialty _____

To properly process purchase orders and for more personal service, mail this coupon to: Dennis Carson, The C.V. Mosby Company, 11830 Westline Industrial Dr., St. Louis, MO 63146. 30 day approval good in U.S. and Canada. PRICES SLIGHTLY HIGHER OUTSIDE U.S. Outside U.S. and Canada, please pay by check or money order in dollar equivalent. ALL PRICES SUBJECT TO CHANGE. Add applicable sales tax.

AMS-938-031-03

Here's what MOSBY'S BOUND VOLUMES can add to your practice!



MOSBY
TIMES MIRROR

Handsomely bound in durable buckram with rich gold stamping, bound journal reference volumes are important to every successful practice.

- gives quick and easy access to important journal articles
- eliminates problems with missing issues or pages
- makes your regular subscription a "working" copy to mark up, tear from, or file
- offers an inexpensive and easy way to guarantee bound reference volumes—prices include shipping

You Must Order Now!
Bound volumes are made up from over-run copies of each journal issue. You never worry about sending out your personal copies. But over-run quantities are limited. We need your order today! Bound volumes are only available to current subscribers and may not be ordered in place of a regular journal subscription. Payment must accompany your order.

Credit Card Orders In The U.S. Call FREE:
1-800-325-4177, ext. 351
(In Missouri, call collect: (314) 872-8370, ext. 351)

YES! Send me the 1987 bound volumes of:

☐ *AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY*

Jan.-June, 1987 (Vol. 156, No. 1-6)
July-Dec., 1987 (Vol. 157, No. 1-6)

Total 1987 Price, Two Volume Set

USA	International
$51.00	$74.00

All orders must include payment. Bound volumes are non-returnable.

My check for $_____ is enclosed.

☐ VISA ☐ MasterCard

Card No._____

Exp. date_____

Name_____
(Please Print)

Address_____

_____

City_____

State_____ Zip_____

Clip and return coupon to: The C.V. Mosby Company, Attn: Bound Volumes, 11830 Westline Industrial Dr., St. Louis, MO 63146. Outside the U.S. please pay by check drawn on a U.S. bank in U.S. dollars or use credit card. **ABS-127**

INDEX TO ADVERTISERS

Allentown Hospital, The
Opportunity Available 60A

Ayerst Laboratories
Permarin 18A,19A,20A

Bemidji Clinic, Ltd.
Opportunity Available 42A

Berlex Laboratories, Inc.
Tri-Levlen 30A,31A,32A

Boston University School of Medicine
Opportunity Available 44A

Ciba Pharmaceutical Company
Estraderm 33A,34A,35A,36A

Cook County Graduate School of Medicine, The
Corporate 26A

Creighton University Medical Center
Opportunity Available 42A

Film-Tec
Informed Consent Systems 42A

Fleisch, Charles M., D.O., FACOG
Opportunity Available 44A

Group Health, Inc.
Opportunity Available 44A

Gyn Cme Fund
Program 47A

Kaiser Permanente Foundation
Opportunity Available 44A

Mary Imogene Bassett Hospital
Opportunity Available 54A

Mead Johnson Laboratories
Estrace 48A,49A,50A
Natalins RX 37A
Ovcon-35 ..... 22A,23A,24A
Ovcon-50 ..... 56A,57A,58A

Medical Equipment Designs, Inc.
HemoCue 10A,11A

Merck Sharp & Dohme
Mefoxin 52A,53A,54A

Michael Reese Health Plan
Opportunity Available 54A

Miles Pharmaceuticals
Mycelex Twin Pack 7A,8A

Mosby, C.V., The
Book 51A

Mount Sinai Medical Center
Opportunity Available 46A

Ortho-Pharmaceutical Corporation
Ortho-Novum 7/7/7 . Third Cover, Fourth Cover

Pennsylvania Hospital
Opportunity Available 59A

Postgraduate Obstetrics & Gynecology
Journal 55A

Permanente Medical Group, Inc., The
Opportunity Available 61A

Rose Medical Center
Opportunity Available 59A

Sinai Hospital of Detroit
Opportunity Available 59A

St. John Hospital
Opportunity Available 60A

St. John's Mercy Medical Center
Conference 26A
Opportunity Available 62A

Stuart Pharmaceuticals
Cefotan 38A,39A,40A

Sunlife OB/GYN Services, Inc.
Opportunity Available 60A

Syntex Laboratories, Inc.
Femstat 41A,43A,45A,46A
Tri-Norinyl ..... Second Cover,1A,2A

Temple University
Opportunity Available 60A

University of Massachusetts, The
Opportunity Available 61A

Upjohn
Provera 3A,4A

While every precaution is taken to insure accuracy, we cannot guarantee against the possibility of an occasional change or omission in the preparation of this index.

ORTHO-NOVUM 7/7/7

TRADEMARK

Tablets

Enhanced patient compliance

(norethindrone/ethinyl estradiol)

Each white tablet contains 0.5 mg norethindrone and 0.035 mg ethinyl estradiol. Each light peach tablet contains 0.75 mg norethindrone and 0.035 mg ethinyl estradiol. Each peach tablet contains 1 mg norethindrone and 0.035 mg ethinyl estradiol. Each green tablet in 28-day regimen contains inert ingredients.

IMPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the product and therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables, and references. The physician should be thoroughly familiar with the complete prescribing information before prescribing the product.

INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

DOSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing .05 mg or less of estrogen.

CONTRAINDICATIONS: Oral contraceptives should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed, abnormal genital bleeding. 7. Oral contraceptive tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who are pregnant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study conducted in the United States found that past use of oral contraceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk may be related to duration of oral contraceptive use. **MYOCARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists following discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the past use. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is discontinued. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. OCULAR LESIONS. There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. CARCINOMA. Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. HEPATIC TUMORS. Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5. USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING. The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. GALLBLADDER DISEASE. Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. HEADACHE. The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. BLEEDING IRREGULARITIES. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. BREAST FEEDING. Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **INFORMATION FOR THE PATIENT: (See Patient Package Insert). DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **CARCINOGENESIS, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS and WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea, usually the most common adverse reaction. Vomiting, occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Revised 12/84

ORTHO-PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869

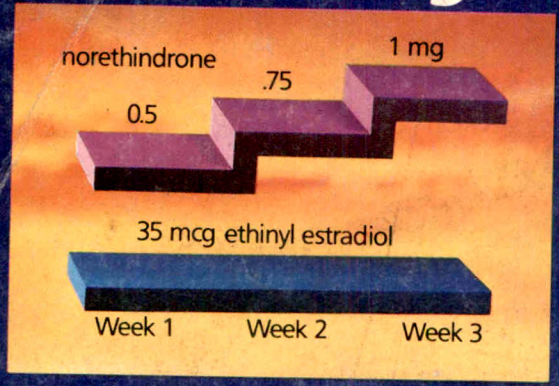
OJ-783

ORTHO

#1 for new start pati

What to look for in a phasic†...Reason 1

Unique, week-by-week design



- ☐ No hormonal drop-off
- ☐ Hormonal doses balanced for endometrial support

7 7 7

Designed for performance

21- and 28-day regimens

ORTHO-NOVUM 7/7/7 Tablets

TRADEMARK

(norethindrone/ethinyl estradiol)

†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.
Please see complete Prescribing Information, a summary of which appears on the preceding page.

ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869

OJ-781

© OPC 1985

ORTHO

February 1987
volume 156, *number* 2

Acc.
16.12.87

American Journal of OBSTETRICS AND GYNECOLOGY

Copyright © 1987 by The C. V. Mosby Company

Editor in Chief
JOHN I. BREWER

Editors
FREDERICK P. ZUSPAN • E. J. QUILLIGAN

Associate Editor
ALBERT B. GERBIE

Official Publication

AMERICAN GYNECOLOGICAL AND OBSTETRICAL SOCIETY
CENTRAL ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
SOCIETY OF OBSTETRICIANS AND GYNAECOLOGISTS OF CANADA
SOUTH ATLANTIC ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
PACIFIC COAST OBSTETRICAL AND GYNECOLOGICAL SOCIETY
AMERICAN BOARD OF OBSTETRICS AND GYNECOLOGY
SOCIETY FOR GYNECOLOGIC INVESTIGATION
SOCIETY OF PERINATAL OBSTETRICIANS

Published by

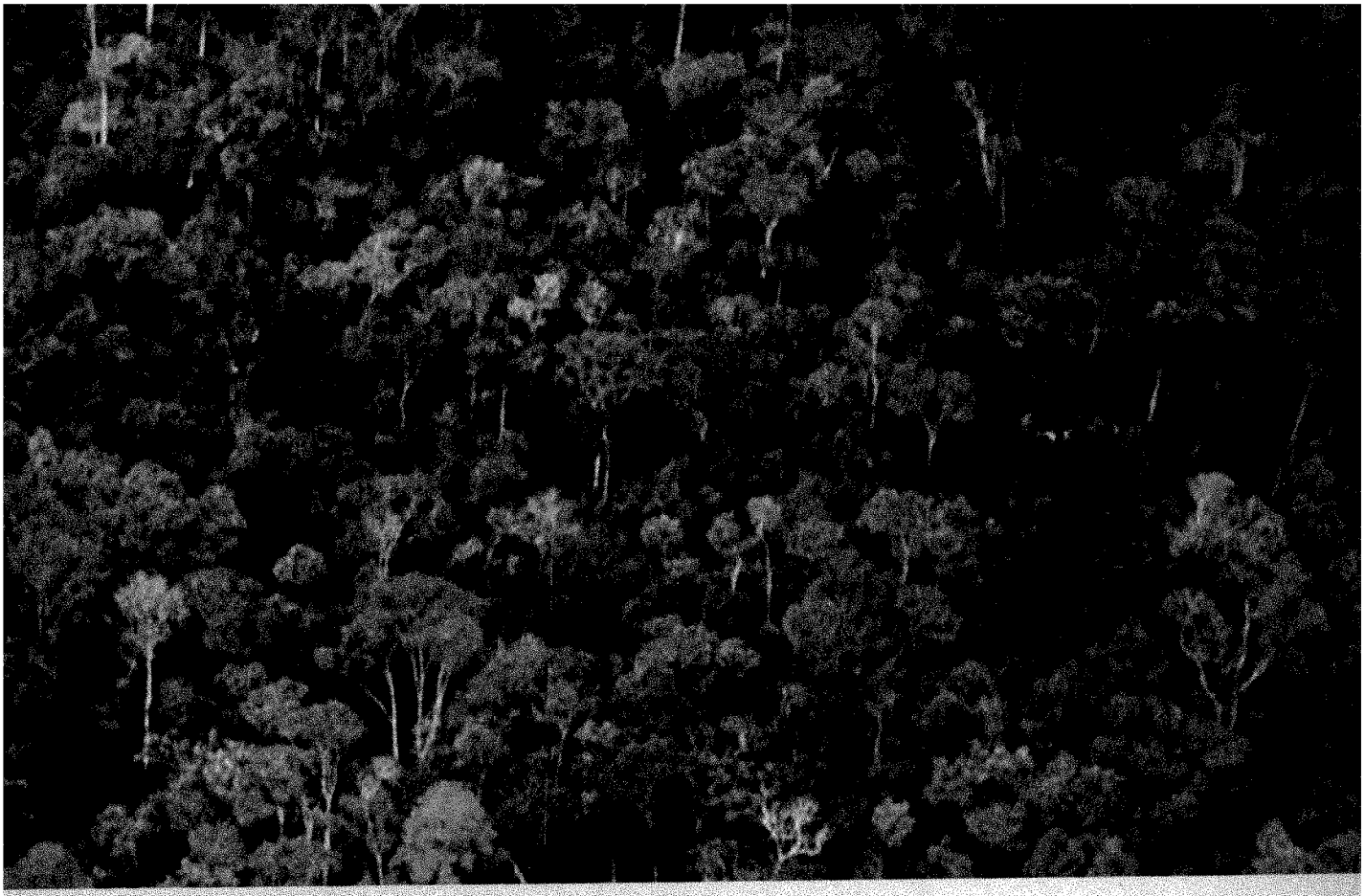
THE C. V. MOSBY COMPANY
St. Louis, Missouri 63146

ISSN 0002-9378



UNIQUE

Full-cycle endometrial support, with no late-cycle progestin increase.



The unique TRI-NORINYL phasing achieves BTB control equivalent to a 1/35 formulation, but with 29% less progestin per cycle. This is, in fact, the greatest progestin reduction of any norethindrone-based multiphasic. TRI-NORINYL lowers progestin where requirements for endometrial support are reduced—with no greater reported BTB than a 1/35 formulation.*

9 DAYS 1.0 MG NORETHINDRONE

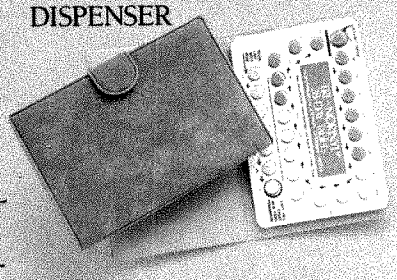
7 DAYS 0.5 MG NORETHINDRONE

5 DAYS 0.5 MG NORETHINDRONE

21 DAYS 0.035 MG ETHINYL ESTRADIOL

PILL DAYS

SUPPLIED IN THE EXCLUSIVE SYNTEX WALLETTE™ PILL DISPENSER



Preferred by patients 3 to 1 over other available packages for convenience, discreetness and compliance.**

THE NATURE OF TRI-NORINYL

*1/35 formulations contain 1.0 mg norethindrone with 0.035 mg ethinyl estradiol. Data available from Syntex Laboratories, Inc.

**Based on a nationwide, independently conducted survey of prospective oral contraceptive patients. Data available from Syntex Laboratories, Inc.

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see brief summary of full prescribing information on next page.

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

©1986 Syntex Laboratories, Inc.

THE NATURE OF TRI-NORINYL®

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

TRI-NORINYL® 21-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)

TRI-NORINYL® 28-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg; 7 inert tablets)

ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications Prevention of pregnancy. *DOSE-RELATED RISK OF THROMBOEMBOLISM.* Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested the persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34 – 5/100,000; ages 35-44 – 33/100,000; ages 45-49 – 140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34 – 1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over – 1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and for MI is further increased by hypertension, hyperlipidemias, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS – RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40 +
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A – Use associated with very high risk.
B – Use associated with high risk.
C – Use associated with moderate risk.
D – Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden, or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema, or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to the registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer, and other studies suggested an increased risk of breast cancer with long-term OC use before age 25. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared to nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. Two other studies suggested an increasing incidence of cervical neoplasms with longer duration of OC use. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OCs with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones – estrogenic and progestational agents – during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase the risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in the results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. One study suggests *Chlamydia trachomatis* infection, the leading cause of nongonococcal P.I.D., is enhanced by OCs. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failure. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions** *GENERAL* 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding from interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, antimigraine preparations, antihistamines, and griseofulvin. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, theophylline, and beta blockers. Toxic liver effects have been reported with a combination of an OC and cyclosporin A. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Warnings. **Adverse Reactions** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps, colitis), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, changes in corneal curvature (steepening), change in weight (increase or decrease) change in cervical erosion and cervical secretion, possible diminution of lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chilblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, vaginitis, porphyria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome), malignant hypertension, acute renal failure. **Information for the Patient** (See Patient Package Insert).

Rev. 6/86

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

A Standard in Progesterone Therapy

Provera® 10 MG TABLETS
(medroxyprogesterone acetate)

WARNING

THE USE OF PROGESTATIONAL AGENTS DURING THE FIRST FOUR MONTHS OF PREGNANCY IS NOT RECOMMENDED.

Progestational agents have been used beginning with the first trimester of pregnancy in an attempt to prevent habitual abortion or treat threatened abortion. There is no adequate evidence that such use is effective and there is evidence of potential harm to the fetus when such drugs are given during the first four months of pregnancy. Furthermore, in the vast majority of women, the cause of abortion is a defective ovum, which progestational agents could not be expected to influence. In addition, the use of progestational agents, with their uterine-relaxant properties, in patients with fertilized defective ova may cause a delay in spontaneous abortion. Therefore, the use of such drugs during the first four months of pregnancy is not recommended.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies including congenital heart defects and limb reduction defects. One study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000.

If the patient is exposed to PROVERA Tablets (medroxyprogesterone acetate) during the first four months of pregnancy or if she becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus.

INDICATIONS: Secondary amenorrhea; abnormal uterine bleeding due to hormonal imbalance in the absence of organic pathology such as fibroids or uterine cancer.

CONTRAINDICATIONS: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy or patients with a past history of these conditions. Liver dysfunction or disease. Known or suspected malignancy of breast or genital organs. Undiagnosed vaginal bleeding. Missed abortion. Known sensitivity to medroxyprogesterone acetate. As a diagnostic test to pregnancy.

WARNINGS: 1. Immediately discontinue administration should any of the following thrombotic disorders occur or be suspected: thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis. **2.** Beagle dogs treated with medroxyprogesterone acetate developed mammary nodules some of which were malignant. Although nodules occasionally appeared in control animals, they were intermittent in nature; whereas the nodules in the drug treated animals were larger, more numerous, persistent, and there were some breast malignancies with metastases. Their significance with respect to humans has not been established. **3.** Discontinue medication pending examination if there is sudden partial or complete loss of vision, onset of proptosis, diplopia, or migraine. If papilledema or retinal vascular lesions occur, withdraw medication. **4.** Detectable amounts of progestin have been identified in the milk of mothers receiving the drug. The effect of this on the nursing infant has not been determined. **5.** Usage in pregnancy is not recommended (see Warning box). **6.** Three major studies in Great Britain and one in this country have shown a statistically significant association between thrombophlebitis, pulmonary embolism, cerebral thrombosis and embolism and the use of oral contraceptives. It has been estimated that users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study indicated that the risk did not persist after discontinuation, and it was not enhanced by long-continued administration.

PRECAUTIONS: A pretreatment physical exam should include special reference to breast and pelvic organs and a Papanicolaou smear. This drug may cause fluid retention; therefore, carefully observe patients with conditions influenced by fluid retention such as epilepsy, migraine, asthma, and cardiac or renal dysfunction. In irregular bleeding per vaginum, bear in mind nonfunctional causes and perform adequate diagnostic measures. Advise pathologist of therapy when submitting relevant specimens. Carefully observe patients with history of psychic depression and discontinue drug if serious depression recurs. Any possible influence of prolonged therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study. Decreased glucose tolerance has been observed in a small percentage of patients on estrogen-progestin combinations; therefore, carefully observe diabetic patients receiving progestin therapy. Age constitutes no absolute limiting factor, although onset of climacteric may be masked. Because of the occasional occurrence of thrombotic disorders (thrombophlebitis, pulmonary embolism, retinal thrombosis, and cerebrovascular disorders) in patients taking estrogen-progestin combinations and since the mechanism is obscure, the physician should be alert to the earliest manifestation of these disorders. (See Patient Information for complete prescribing information.)

ADVERSE REACTIONS: Pregnancy: (see Warning box); **Breast:** rare reports of breast tenderness or galactorrhea; **Skin:** sensitivity reactions including pruritus, urticaria, edema and generalized rash; acne, alopecia and hirsutism in a few patients; **Thromboembolic Phenomena** including thrombophlebitis and pulmonary embolism.

The following adverse reactions have been observed in women taking progestins including medroxyprogesterone acetate: breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; edema; change in weight; changes in cervical erosion and secretions; cholestatic jaundice; rash (allergic) with and without pruritus; mental depression; anaphylaxis and anaphylactoid reactions; pyrexia; insomnia; nausea and somnolence.

A statistically significant association has been demonstrated between use of estrogen-progestin combination drugs and the serious adverse reactions of thrombophlebitis, pulmonary embolism and cerebral thrombosis and embolism. Therefore, patients on progestin therapy should be carefully observed.

Although available evidence is suggestive, a relationship has been neither confirmed nor refuted for the association of the serious adverse reaction of neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been observed in patients receiving estrogen-progestin combination drugs: rise in blood pressure in susceptible individuals; premenstrual-like syndrome; changes in libido; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; fatigue; backache; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; and itching.Therefore, observe patients on progestin therapy carefully.

The following laboratory results may be altered by the use of estrogen-progestin combination drugs: increased sulfobromophthalein retention and other hepatic function tests; coagulation tests (increase in prothrombin factors VII, VIII, IX and X); metyrapone test; pregnanediol determination; thyroid function tests (increase in PBI, and butanol extractable protein bound iodine and decrease in T^3 uptake values).

Caution: Federal law prohibits dispensing without prescription.

MED B-13-S

Upjohn 100

A Century of Caring
1886·1986

March 1986
J-6144

© 1986 The Upjohn Company

The Upjohn Company, Kalamazoo, MI 49001

A Standard



Provera® 10 MG TABLETS
(medroxyprogesterone acetate)

A Standard in Progesterone Therapy

Please see adjacent page for brief summary of prescribing information.

MOSBY

American Journal of Obstetrics and Gynecology

Founded in 1920

Copyright © 1987 by The C. V. Mosby Company

Contents

February
1987

This issue contains Transactions of the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, a Clinical Section beginning on page 425, and a Symposium on the Role of β-Lactamase Inhibitor Combinations in the Management of Obstetric-Gynecologic Infections beginning on page 491.

TRANSACTIONS OF THE FORTY-SECOND ANNUAL MEETING OF THE SOCIETY OF OBSTETRICIANS AND GYNAECOLOGISTS OF CANADA

Psychological characteristics of in vitro fertilization participants 269
M. T. Hearn, Ph.D., A. A. Yuzpe, M.D., S. E. Brown, M.D., and R. F. Casper, M.D.
London, Ontario, Canada

Detecting changes in functional ability in women with premenstrual syndrome 275
Barbara W. Posthuma, O.T.(C), M.Ed., Martin J. Bass, M.D., Shelley B. Bull, Ph.D., and Jeffrey A. Nisker, M.D.
London, Ontario, Canada

(Contents continued on page 6A)

Vol. 156, No. 2, February 1987. The American Journal of Obstetrics and Gynecology (ISSN 0002-9378) is published monthly by The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146. POSTMASTER: Send address changes to The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

1987 Annual subscription rates	U.S.A.	International (surface mail) All regions	International (airmail)* Canada & Mexico	Region 1	Region 2	Region 3
Institutions†	$102.00	$121.00	$142.55	$153.60	$183.85	$214.10
Individuals‡	$ 59.50	$ 78.50	$100.05	$111.10	$141.35	$171.60
Students, residents‡	$ 40.00	$ 59.00	$ 80.55	$ 91.60	$121.85	$152.10

Single copies are $6.50. Remittances should be made by check, draft, or post office or express money order, in U.S. funds drawn through a U.S. bank, payable to this JOURNAL. Claims for missing issues will be serviced only within six months of cover date. Single copy prices will be charged on missing issue claims older than six months from cover date.

*Airmail breakdown—Domestic: First-class and Priority rates for the U.S. and possessions are available upon request. *Region 1:* Central America, islands, and mainland colonies of European countries in The Americas. *Region 2:* South America, Europe, Egypt, Africa (bordering the Mediterranean). *Region 3:* Asia, Australasia, Africa (other than Mediterranean), Middle East, Far East, The Pacific, U.S.S.R. (and constituent Republics).

†Institutional (multiple-reader) subscriptions are available to public and private libraries, schools, hospitals, and clinics; city, county, state, provincial, and national government bureaus and departments; and all commercial and private institutions and organizations.

‡Individual subscriptions and all student-rate subscriptions must be in the names of, billed to, and paid by individuals. All student-rate requests must indicate training status and name of institution.

Subscriptions may begin at any time.

Second-class postage paid at St. Louis, Missouri, and additional mailing offices. Printed in the U.S.A. Copyright © 1987 by The C. V. Mosby Company.

Contents continued from page 5A

Endocrine changes after laparoscopic ovarian cautery in polycystic ovarian syndrome 279
Ellen Greenblatt, M.D., and Robert F. Casper, M.D.
London, Ontario, Canada

Human chorionic gonadotropin increase in normal early pregnancy 286
Salim Daya, M.B., Ch.B.
Hamilton, Ontario, Canada

Prevalence of antibody to *Chlamydia trachomatis* in spontaneous abortion and infertility 291
P. A. Quinn, Ph.D., M. Petric, Ph.D., M. Barkin, M.D., J. Butany, M.D., C. Derzko, M.D., M. Gysler, M.D., K. I. Lie, M.D., A. B. Shewchuck, M.D., J. Shuber, M.D., E. Ryan, M.D., and M. L. Chipman, M.A.
Toronto, Ontario, Canada

Infertility surgery for pelvic inflammatory disease: Success rates after salpingolysis and salpingostomy 296
Mark Carey, M.D., and Stan Brown, M.D.
London, Ontario, Canada

Temporal variation in rates of cesarean section for dystocia: Does "convenience" play a role? 300
William Fraser, M.D., Robert H. Usher, M.D., Frances H. McLean, B.Sc.N., Carol Bossenberry, B.N., Mary Ellen Thomson, Ph.D., Michael S. Kramer, M.D., L. Paul Smith, M.D., and Hugh Power, M.D.
Montreal, Quebec, Canada

Maternal age and primary cesarean section rates: A multivariate analysis 305
Michelle Martel, M.D., Sholom Wacholder, Ph.D., Abby Lippman, Ph.D., John Brohan, B.Sc., and Emily Hamilton, M.D.C.M.
Montreal, Quebec, Canada

Prenatal diagnosis and management of congenital defects of the anterior abdominal wall 308
M. Sermer, M.D., R. J. Benzie, M.D., L. Pitson, M.D., M. Carr, M.D., and M. Skidmore, M.D.
Toronto, Ontario, Canada

Congenital heart disease in pregnancy: Short- and long-term implications 313
J. Shime, M.D., E. J. M. Mocarski, M.Sc., M.D., D. Hastings, M.D., G. D. Webb, M.D., and P. R. McLaughlin, M.D.
Toronto, Ontario, Canada

(Contents continued on page 9A)

Mycelex® Twin Pack
(clotrimazole)

One-day therapy

Mycelex®-G 500 mg
(clotrimazole) Vaginal Tablet

The shortest regimen for vulvovaginal candidiasis

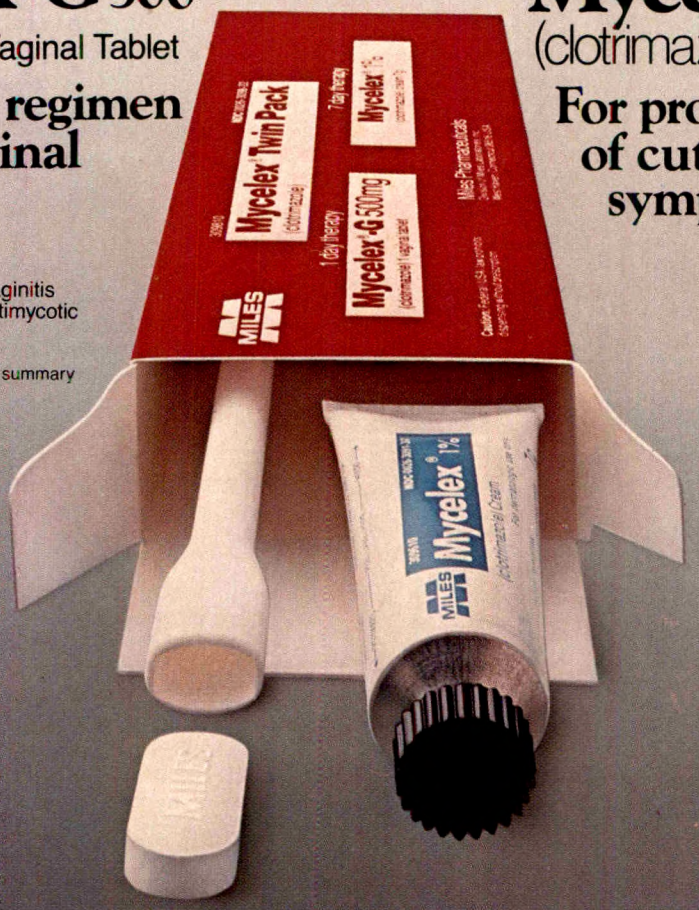
In the case of severe vulvovaginitis due to candidiasis, longer antimycotic therapy is recommended.

Please see following page for brief summary of prescribing information.

Seven-day therapy

Mycelex® 1%
(clotrimazole) Cream

For prompt relief of cutaneous symptoms



Mycelex® Twin Pack

Miles Pharmaceuticals
Division of Miles Laboratories, Inc.
West Haven, Connecticut 06516 USA

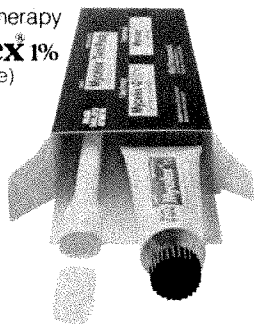
© 1986 Miles Pharmaceuticals Division of Miles Laboratories, Inc.

MILES
Miles Pharmaceuticals

Mycelex® Twin Pack
(clotrimazole)

One-day therapy
Mycelex®-G 500mg
(clotrimazole) Vaginal Tablet

Seven-day therapy
Mycelex® 1%
(clotrimazole)
Cream



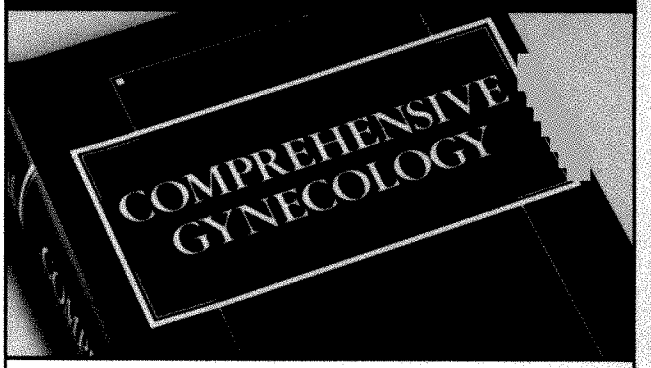
Indications
MYCELEX®-G 500 mg Vaginal Tablets are indicated for the local treatment of vulvovaginal candidiasis when one-day therapy is felt warranted. In the case of severe vulvovaginitis due to candidiasis, longer antimycotic therapy is recommended.
MYCELEX® CREAM is indicated for the topical treatment of dermal infections due to *Candida albicans*.
Contraindications
MYCELEX-G 500 mg Vaginal Tablets and MYCELEX CREAM are contraindicated in women who have shown hypersensitivity to any of their components.
Warnings
MYCELEX-G 500 mg Vaginal Tablets: none. MYCELEX CREAM is not for ophthalmic use.
Precautions
MYCELEX-G 500 mg Vaginal Tablets: If there is a lack of response to the Vaginal Tablets, appropriate microbiological studies should be repeated to confirm the diagnosis and rule out other pathogens before instituting another course of antimycotic therapy.
MYCELEX CREAM: If irritation or sensitivity develops with the use of Mycelex Cream, treatment should be discontinued and appropriate therapy instituted.
Carcinogenesis
MYCELEX-G 500 mg Vaginal Tablets: No long-term studies in animals have been performed to evaluate the carcinogenic potential of 500 mg Vaginal Tablets intravaginally. A long-term study in rats (Wistar strains) where clotrimazole was administered orally provided no indication of carcinogenicity.
Usage in Pregnancy: Pregnancy Category B:
MYCELEX-G 500 mg Vaginal Tablets: The disposition of ^{14}C-clotrimazole has been studied in humans and animals. Clotrimazole is poorly absorbed following intravaginal administration to humans, whereas it is rather well absorbed after oral administration.
In clinical trials, use of vaginally applied clotrimazole in pregnant women in their second and third trimesters has not been associated with ill effects. There are, however, no adequate and well-controlled studies in pregnant women during the first trimester of pregnancy.
Because animal reproduction studies are not always predictive of human response, this drug should be used only if clearly indicated during the first trimester of pregnancy.
MYCELEX CREAM: In the first trimester of pregnancy, Mycelex Cream should be used only when considered essential to the welfare of the patient.
Adverse Reactions
MYCELEX-G 500 mg Vaginal Tablets: Of 297 patients in double-blind studies with the 500 mg vaginal tablet, 3 of 149 patients treated with active drug and 3 of 148 patients treated with placebo reported complaints during therapy that were possibly drug related. In the active drug group, vomiting occurred in one patient, vaginal soreness with coitus in another, and complaints of vaginal irritation, itching, burning and dyspareunia in the third patient. In the placebo group, clitoral irritation occurred in one patient and dysuria, described as remotely related to drug, in the other. A third patient in the placebo group developed bacterial vaginitis which the investigator classed as possibly related to drug.
MYCELEX CREAM: The following reactions have been reported in connection with the use of this cream: erythema, stinging, blistering, peeling, edema, pruritus, urticaria, and general irritation of the skin.

Issued, July 1986

Miles Pharmaceuticals
Division of Miles Laboratories, Inc.
West Haven, Connecticut 06516 USA

MILES
Miles Pharmaceuticals

Innovation Plus Expertise!



COMPREHENSIVE GYNECOLOGY

By William Droegemueller, M.D.; Arthur L. Herbst, M.D.; Daniel R. Mishell, Jr., M.D.; and Morton A. Stenchever, M.D.

Innovative enough to emphasize the psychosocial treatment of your patient, with the expertise to cover a full range of diseases and physical problems — this combination brings you the first, all new comprehensive gynecology text in years! Comprehensive discussions on:

- the most current treatments available
- psychosocial approaches to the troubled patient
- family planning and pregnancy termination
- breast disease and sexually transmitted diseases, including AIDS in women
- rape, incest and other physical abuse

The Key Terms and Definitions that begin each chapter and the synopsis of Key Points at the end provide a quick review. Sophisticated graphics highlight pertinent data. Written by four well-known specialists who share their experience and expertise, COMPREHENSIVE GYNECOLOGY presents state-of-the-art information you'll refer to again and again.

July, 1987. Approx. 1,000 pages with 800 illustrations. (Book Code: 01929) About $60.00 (U.S.); $82.95 (Can.)

In a hurry to order?
Call FREE 800-221-7700, ext. 15A.
Our hotline is open 24 hours a day, 7 days a week. In Canada call 416-298-1588.
AMS-969-031

MOSBY
TIMES MIRROR

Yes! Send me a copy of Droegemueller's COMPREHENSIVE GYNECOLOGY (01929) on 30-day approval priced about $60.00 (U.S.); $82.95 (Can.)

Save money! Enclose payment with your order or charge it to your credit card. Mosby will pick up the tab for shipping and handling.

☐ Payment enclosed
Charge my credit card: ☐ MasterCard ☐ VISA

Card # _____ Exp. date _____

Signature _____

Business phone (_____) _____

☐ Bill me, plus shipping and handling.

Name _____

Address _____

City _____

State _____ Zip _____

☐ Add me to your mailing list. (No purchase necessary)

Specialty _____

To properly process Purchase Orders and for more personal service, mail this coupon to: Dennis Carson, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, MO 63146. 30-day approval good in U.S. and Canada. Outside U.S. and Canada, please pay in dollar equivalent. ALL PRICES SUBJECT TO CHANGE. Add applicable sales tax.

AMS-969-031-03

Contents continued from page 6A

External vibratory acoustic stimulation near term: Fetal heart rate and heart rate variability responses 323
Robert Gagnon, M.D., Cora Hunse, R.N., Lesley Carmichael, B.Sc., Fraser Fellows, M.D., and John Patrick, M.D.
London, Ontario, Canada

Psychological adaptation to pregnancy in childless women more than 35 years of age 328
G. Erlick Robinson, M.D., David M. Garner, Ph.D., Douglas J. Gare, M.D., and Belinda Crawford, B.Sc.
Toronto, Ontario, Canada

Shoulder dystocia: Predictors and outcome 334
Susan J. Gross, M.D., Jerry Shime, M.D., and Dan Farine, M.D.
Toronto, Ontario, Canada

Cervical ripening and labor induction with prostaglandin E_2 gel: A placebo-controlled study 336
Paul Bernstein, M.D., Nicholas Leyland, M.D., Paul Gurland, M.D., and Douglas Gare, M.D.
Toronto, Ontario, Canada

Clinical utility of multiple-dose administration of prostaglandin E_2 gel 341
Thomas Mainprize, M.D., Carl Nimrod, M.B., Gail Dodd, R.N., and David Persaud, M.Sc.
Calgary, Alberta, Canada

Immunosuppressor factor(s) produced by decidua-associated suppressor cells: A proposed mechanism for fetal allograft survival 344
Salim Daya, M.B., Ch.B., Kenneth L. Rosenthal, Ph.D., and David A. Clark, M.D., Ph.D.
Hamilton, Ontario, Canada

Fetal foot length as a predictor of gestational age 350
Brian M. Mercer, M.D., Suzi Sklar, R.D.M.S., Anahita Shariatmadar, M.Sc., Martin S. Gillieson, M.D., and Mary E. D'Alton, M.D.
Ottawa, Ontario, Canada

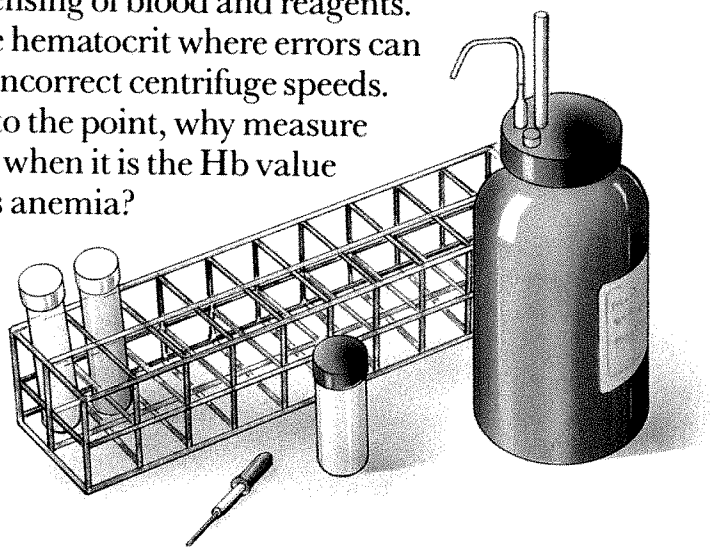
The effect of hydralazine on placental perfusion in the spontaneously hypertensive rat 356
Jeffrey Lipshitz, M.D., Robert A. Ahokas, Ph.D., and Samuel L. Reynolds, M.S.
Calgary, Alberta, Canada, and Memphis, Tennessee

(Contents continued on page 12A)

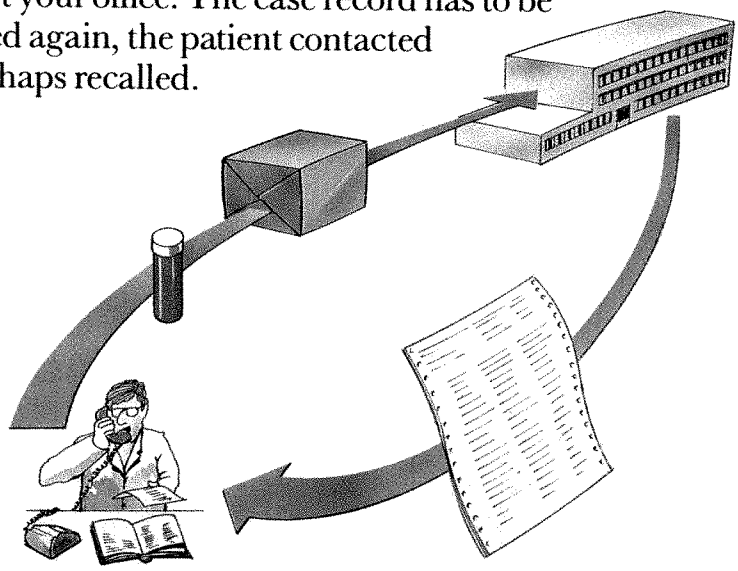
Hemoglobin determination, traditional procedures.

Traditional in-office Hb testing involves messy reagents, pipettes, test tubes, etc – not to mention instrument calibration and zero setting. A complex and time consuming procedure with high error potential, especially in the dispensing of blood and reagents.

Or take hematocrit where errors can arise from incorrect centrifuge speeds. And more to the point, why measure hematocrit when it is the Hb value that defines anemia?



And what if you use an outside laboratory instead? When the test results finally arrive the patient has long since left your office. The case record has to be extracted again, the patient contacted and perhaps recalled.



Sales and service outside the U.S.:
Denmark: Ercopharm A/S. Tel. (02) 89 08 89. **Finland:** Oy Tamro Ab. Tel. 90-544 011. **Norway:** A/S Saba Medical. Tel. (02) 64 47 30. **Iceland:** G Olafsson h/f. Tel. 01-841 66. **Greece:** Pharmaserve SACI. Tel. 01-6726 380. **Ireland:** Accu-Science (Ireland) Ltd. Tel. 045-603 91. **Italy:** Labanalysis srl. Tel. (06) 45 03 918. **The Netherlands:** UCB Farma Nederland BV. Tel. 076-87 92 22. **Switzerland:** Baumann-Medical. Tel. 01-932 30 92. **France:** TLM Laboratoire SARL. Tel. (1) 39 65 69 09. **Great Britain:** Clandon Scientific Ltd. Tel. (0252) 51 47 11/5. **Australia & New Zealand:** Medipac Pty Ltd. Tel. 043-28 20 26. **Pakistan:** S. Ejazuddin & Co. Tel. (Karachi) 22 44 20. **Singapore:** Transmedic Pte Ltd. Tel. 274 66 33. **Malaysia:** Translab (M) Sdn Bhd. Tel. 03-78 10 44. **Indonesia:** Berca Indonesia Pt. Tel. 021-34 38 86. **Hongkong & Macao:** Science International. Tel. 05-43 74 42. **Taiwan:** Transmedic Co Ltd. Tel. 02-981 5343. **South Korea:** Poong Nam Corp. Tel. (02) 557-6947.

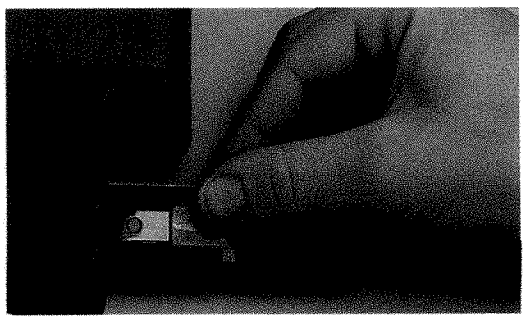
The new 60-second HemoCue procedure.



1. *Obtain a blood sample by finger stick.*



2. *The HemoCue microcuvette draws an exact amount of blood.*



3. *Insert the microcuvette into the HemoCue photometer.*



4 *Result displayed automatically within*

The secret of the HemoCue Hb test system lies in the unique disposable microcuvette, which serves as pipette, test tube and measuring vessel in one. Containing a dry reagent it self-fills with blood by capillary action to produce a precise chemical reaction.

The HemoCue photometer is fully automated. The only control is the ON/OFF switch! No liquid reagents are used. Zero setting and functional adjustments are performed automatically between tests. It also automatically compensates for turbidity in the sample!

Thanks to HemoCue you can now get **accurate** Hb test results, truly stat, while the patient is still in your office.

For more information on this new and economical test, call 800-323-1674 (CA 714-859-7779).

HemoCue®

Developed and manufactured by
Leo Diagnostics AB, S-251 09 Helsingborg, Sweden.

Marketing and technical service in the USA exclusively through
Medical Equipment Designs, Inc.
23521 Ridge Route Dr. Suite A, Laguna Hills, CA 92653

Contents continued from page 9A

Fetal oxygen extraction: Comparison of the human and sheep 360
D. Rurak, D.Phil., R. Selke, M.D., M. Fisher, M.D., S. Taylor, A.H.T., and B. Wittmann, M.D.
Vancouver, British Columbia, Canada

Endometrial carcinoma occurring in patients under the age of 45 years 366
J. D. Jeffery, M.D., R. Taylor, M.D., D. I. Robertson, M.D., and G. C. E. Stuart, M.D.
Calgary, Alberta, Canada

Uterine sarcoma: An analysis of 74 cases 370
Allan L. Covens, M.D., Jeffrey A. Nisker, M.D., William B. Chapman, M.D., and H. Hugh Allen, M.D.
London, Ontario, Canada

Laser surgery for vulvar intraepithelial neoplasia: Principles and results 374
V. C. Wright, M.D., and Eleanor Davies, M.D.
London, Ontario, Canada

Chorioamnionitis: Its association with pregnancy outcome and microbial infection 379
P. A. Quinn, Ph.D., J. Butany, M.D., J. Taylor, M.D., and W. Hannah, M.D.
Toronto, Ontario, Canada

Massive genital and vaginal vault prolapse treated by abdominal-vaginal sacropexy with use of Marlex mesh: Review of the literature 387
Harold P. Drutz, M.D., and Lily S. Cha, M.D.
Toronto, Ontario, Canada

Sacrococcygeal teratoma: Prenatal diagnosis and management 393
Susan J. Gross, M.D., Ronald J. Benzie, M.B., Ch.B., Mathew Sermer, M.D., Martin B. Skidmore, M.B., and Stephanie R. Wilson, M.D.
Toronto, Ontario, Canada

Human chorionic gonadotropin, prolactin, estriol, and dehydroepiandrosterone sulfate concentrations in cord blood of premature and term newborn infants: Relationship to the sex of the neonate 396
Basil Ho Yuen, M.B., Ch.B., and Everett K. Mincey, Ph.D.
Vancouver, British Columbia, Canada

Differing concentrations of human chorionic gonadotropin and prolactin in the cyst fluid of hydatidiform mole and in amniotic fluid 400
Basil Ho Yuen, M.B., Ch.B.
Vancouver, British Columbia, Canada

(Contents continued on page 14A)

FOR VULVOVAGINAL CANDIDIASIS

Think Cream



Contents continued from page 12A

Doppler ultrasound prediction of fetal outcome in twin pregnancies 402
C. Nimrod, M.B., D. Davies, R.D.M.S., J. Harder, M.D., C. Dempster, R.D.M.S., G. Dodd, R.N., N. McDicken, Ph.D., and S. Nicholson, M.D.
Calgary, Alberta, Canada

Congenital and nosocomial sepsis in infants born in a regional perinatal unit: Cause, outcome, and white blood cell response 407
Arne Ohlsson, M.D., and Mary Vearncombe, M.D.
Toronto, Ontario, Canada

Postmenopausal estrogen and androgen replacement and lipoprotein lipid concentrations 414
Barbara B. Sherwin, Ph.D., Morrie M. Gelfand, M.D., Reuben Schucher, Ph.D., and Joseph Gabor, M.Sc.
Montreal, Quebec, Canada

Effects of adenosine 3′,5′-cyclic monophosphate, forskolin, and cholera toxin on hormone production in human term placental cells 420
Fuzhen Zhou, Basil Ho Yuen, M.B., and Peter C. K. Leung, Ph.D.
Vancouver, British Columbia, Canada

CLINICAL SECTION

Clinical Opinion

Psychosis and pregnancy: Some new ethical and legal dilemmas for the physician 425
E. Earlene Dal Pozzo, M.D., and Frank H. Marsh, J.D., Ph.D.
Denver, Colorado

Clinical Articles

Characteristics of menopausal women seeking assistance 428
Evelyn Anderson, R.N., Ph.D., Sonia Hamburger, B.A., James H. Liu, M.D., and Robert W. Rebar, M.D.
La Jolla, California

Tocolysis with oral magnesium 433
Rick W. Martin, M.D., Donald K. Gaddy, M.D., James N. Martin, Jr., M.D., John A. Lucas, M.D., Winfred L. Wiser, M.D., and John C. Morrison, M.D.
Jackson, Mississippi

(Contents continued on page 16A)

FOR VULVOVAGINAL CANDIDIASIS

Think 3 Days*



Contents continued from page 14A

Maternal hypothermia: An unusual complication of magnesium sulfate therapy 435
John F. Rodis, M.D., Anthony M. Vintzileos, M.D., Winston A. Campbell, M.D., Jeffrey L. Deaton, M.D., and David J. Nochimson, M.D.
Farmington, Connecticut

Plasma progesterone concentration in women with and without adenocarcinoma of the endometrium 437
Andrew P. Bond, M.D., Michael J. Diver, M.Sc., and J. Collinson Davis, M.D.
Liverpool, England

Predictive value of CA-125 levels in advanced ovarian cancer 440
Bruce Potsner, M.D., and Thomas G. Day, Jr., M.D.
Louisville, Kentucky

Sonographic determination of fetal weights in diabetic pregnancies 441
Carol B. Benson, M.D., Peter M. Doubilet, M.D., Ph.D., and Daniel H. Saltzman, M.D.
Boston, Massachusetts

Ureteral obstruction as a complication of the Burch colposuspension procedure: Case report 445
Gerald B. Applegate, M.D., Katherine M. Bass, M.D., and Carolyn J. Kubik, M.D.
Pittsburgh, Pennsylvania

Vaginal Gram stain as an immediate detector of group B streptococci in selected obstetric patients 446
Steven M. Feld, M.D., and John T. Harrigan, M.D.
New Brunswick, New Jersey

C-reactive protein: A limited test for managing patients with preterm labor or preterm rupture of membranes? 449
J. M. Ernest, M.D., Melissa Swain, R.N., Steven M. Block, M.D., Lewis H. Nelson, M.D., Christos G. Hatjis, M.D., and Paul J. Meis, M.D.
Winston-Salem, North Carolina

Comparison of phospholipids in vaginal and amniocentesis specimens of patients with premature rupture of membranes 454
David C. Shaver, M.D., Joseph A. Spinnato, M.D., David Whybrew, M.S., W. Keith Williams, M.D., and Garland D. Anderson, M.D.
Memphis, Tennessee

Acute *Haemophilus influenzae* chorioamnionitis associated with intact amniotic membranes 458
Hung N. Winn, M.D., and Charles C. Egley, M.D.
Peoria, Illinois

(Contents continued on page 19A)

FOR VULVOVAGINAL CANDIDIASIS

Think

vaginal cream 2.0%

Femstat®

(butoconazole nitrate)





FAST, SOOTHING RELIEF
Complete coverage–
and minimal vaginal leakage![1]

3-DAY THERAPY
As effective as miconazole cream–
in only 3 days' time!

3 DISPOSABLE APPLICATORS
Patient convenience and satisfaction–
for enhanced compliance!

*In non-pregnant patients, 3-day treatment is recommended. Treatment may be extended an additional 3 days, if necessary.

1. Droegemueller W, Adamson DG, Brown D et al: Three-day treatment with butoconazole nitrate for vulvovaginal candidiasis. *Obstet Gynecol* 64:530-534, 1984.

Please see brief summary of prescribing information on next page.

The Only 3-Day Cream*

Think

vaginal cream 2.0%

Femstat®

(butoconazole nitrate)

The Only 3-Day Cream

Brief Summary

INDICATIONS:
FEMSTAT® (butoconazole nitrate) vaginal cream 2.0% is indicated for local treatment of vulvovaginal mycotic infections caused by Candida species. Confirm the diagnosis by KOH smears and/or cultures. FEMSTAT can be used with oral contraceptives and antibiotics. It is effective in non-pregnant women and during the second and third trimesters of pregnancy.

CONTRAINDICATIONS:
FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

PRECAUTIONS:
General:
If clinical symptoms persist, repeat microbiological tests to rule out other pathogens and confirm the diagnosis. Discontinue drug if sensitization or irritation occurs.

Information for the Patient:
Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis:
Animal studies have not been done.

Mutagenesis:
Mutagenicity studies were negative.

Impairment of Fertility:
Animal studies showed no impairment of fertility.

Pregnancy Category C:
Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers:
Use with caution.

Pediatric Use:
Safety and efficacy have not been established.

ADVERSE REACTIONS:
Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

DOSAGE AND ADMINISTRATION:
Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

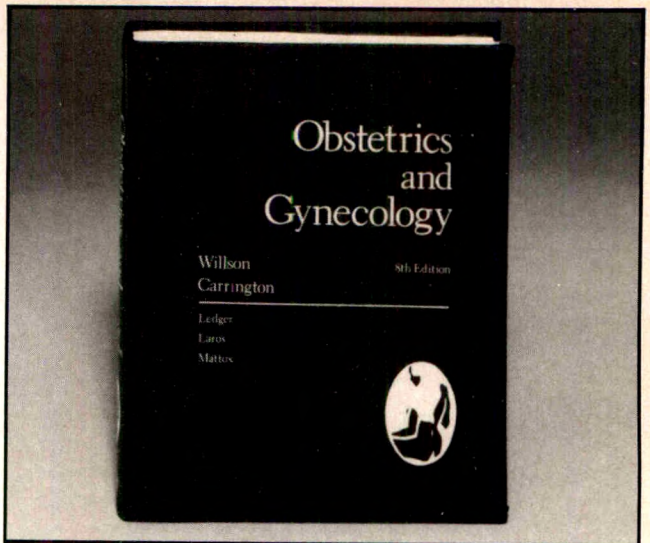
CAUTION:
Federal law prohibits dispensing without prescription.

December 1985

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

© 1986 Syntex Laboratories, Inc.



New 8th Edition! OBSTETRICS AND GYNECOLOGY

Edited by J. Robert Willson, M.D. and Elsie Reid Carrington, M.D.; Associate Editors Russell K. Laros, Jr., M.D.; William J. Ledger, M.D.; and John H. Mattox, M.D.; with 4 contributors.

In a review of the 7th edition, JAMA said that it "maintains excellence especially in general obstetrics" and "fulfills its purpose as a useful text for various levels of students of obstetrics and gynecology." This new edition, with the addition of two new editors, continues the tradition! Emphasis is on treatment of the "total woman," following the normal life-cycle—birth through pregnancy.

- easy-to-read format facilitates Board review
- a new chapter on androgen disorders
- infertility, endocrinology and endometriosis
- ectopic pregnancy, abortion and infectious disease
- radiologic studies in pregnant women

April, 1987. Approx. 768 pages, 562 illustrations. (Book Code: 05650) About $44.95 (U.S.); $62.25 (Can.)

In a hurry to order?
Call FREE 800-221-7700, ext. 15A.
Our hotline is open 24 hours a day, 7 days a week. In Canada call 416-298-1588.

MOSBY
TIMES MIRROR

AMS-970-031

Yes! Send my 30-day on-approval copy of:
Willson's OBSTETRICS AND GYNECOLOGY (05650) about $44.95 (U.S.); $62.25 (Can.)

Save money! Enclose payment with your order or charge it to your credit card. Mosby will pick up the tab for shipping and handling.

☐ Payment enclosed

Charge my credit card: ☐ MasterCard ☐ VISA

Card # _____ Exp. date _____

Signature _____

Business phone (_____) _____

☐ Bill me, plus shipping and handling.

Name _____

Address _____

City _____

State _____ Zip _____

☐ Add me to your mailing list. (No purchase necessary)

Specialty _____

To properly process Purchase Orders and for more personal service, mail this coupon to: Dennis Carson, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, MO 63146. 30-day approval good in U.S. and Canada. Outside U.S. and Canada, please pay in dollar equivalent. ALL PRICES SUBJECT TO CHANGE. Add applicable sales tax.

AMS-970-031-03

Contents continued from page 16A

Maternal serum α-fetoprotein, age, and Down syndrome risk 460
Glenn E. Palomaki, B.S., and James E. Haddow, M.D.
Scarborough, Maine

Delivery of fertility control services by male and female obstetrician-gynecologists 464
Carol S. Weisman, Ph.D., Constance A. Nathanson, Ph.D., Martha Ann Teitelbaum, M.P.H., Ph.D., Gary A. Chase, Ph.D., and Theodore M. King, M.D., Ph.D.
Baltimore, Maryland

Three-contrasts method: An ultrasound technique for monitoring transcervical operations 469
Bao-Liang Lin, M.D., Yoshiyuki Iwata, M.D., Naohiko Miyamoto, M.D., and Shigeru Hayashi, M.D.
Kawasaki, Japan

Prenatal ultrasonic diagnosis of multiple bowel atresias 472
M. Amanda Skoll, M.D., Gerald P. Marquette, M.D., and Emily F. Hamilton, M.D.
Montreal, Quebec, Canada

Effect of naloxone on fetal behavior near term 474
Domenico Arduini, M.D., Giuseppe Rizzo, M.D., Sergio Dell'Acqua, M.D., Salvatore Mancuso, M.D., and Carlo Romanini, M.D.
Rome and Ancona, Italy

Immunocytochemistry of human chorionic gonadotropin in human chorionic villi 479
Kunshige Hamasaki, M.D., Yasushi Okamura, M.D., Hiroshi Ueda, Ph.D., Hirohiko Kagawa, Ph.D., and Sunao Fujimoto, M.D.
Kitakyushu, Japan

Nonsurgical therapy to preserve oviduct function in patients with tubal pregnancies 484
Kihyoe Ichinoe, M.D., Norio Wake, M.D., Naoko Shinkai, M.D., Yoshihiro Shiina, M.D., Yukio Miyazaki, M.D., and Toshinobu Tanaka, M.D.
Sapporo, Japan

ANNOUNCEMENT

Position Statement on Gestational Diabetes Mellitus 488

(Contents continued on page 20A)

Contents continued from page 19A

ITEMS

Items 490

SYMPOSIUM ON THE ROLE OF β-LACTAMASE INHIBITOR COMBINATIONS IN THE MANAGEMENT OF OBSTETRIC-GYNECOLOGIC INFECTIONS

Microbiology of the female genital tract 491
Ronald S. Gibbs, M.D.
San Antonio, Texas

A review of the role of β-lactamase—producing bacteria in obstetric-gynecologic infections 495
David A. Eschenbach, M.D.
Seattle, Washington

Induction of β-lactamase enzymes: Clinical applications for the obstetric-gynecologic patient 504
David L. Hemsell, M.D.
Dallas, Texas

Ampicillin/sulbactam versus metronidazole-gentamicin in the treatment of soft tissue pelvic infections 507
William R. Crombleholme, M.D., Marilyn Ohm-Smith, Marilyn O. Robbie, Vicki DeKay, and Richard L. Sweet, M.D.
San Francisco, California

Infectious morbidity in gynecologic cancer 513
Doris C. Brooker, M.D., John E. Savage, M.D., Leo B. Twiggs, M.D., Leon L. Adcock, M.D., Konald A. Prem, M.D., and Christine C. Sanders, Ph.D.
Minneapolis, Minnesota, and Omaha, Nebraska

CORRESPONDENCE

Surveillance of twin pregnancy with umbilical arterial velocimetry J. W. K. Ritchie, M.D., and Lesley McCowan, M.B. *Toronto, Ontario, Canada* 521

Reply George Farmakides, M.D., and Harold Schulman, M.D. *Mineola, Long Island, New York* 521

Additional statistical analysis of antenatal phenobarbital for prevention of neonatal intracerebral hemorrhage Robert V. Jarrett, D.O., and Glen D. Jordan, M.D. *Tacoma, Washington* 521

Reply Seetha Shankaran, M.D., Eugene E. Cepeda, M.D., and Ronald L. Poland, M.D. *Detroit, Michigan* 522

Contents

Intrapartum fetal heart rate monitoring D. D. Mathews, M.D. *Chatham, Kent, England* 523

Reply Dermot MacDonald, Adrian Grant, Margaret Sheridan-Pereira, Peter Boylan, and Iain Chalmers *Oxford, England* 523

Transcervical chorionic villus sampling R. H. T. Ward, B. Modell, and M. Petrous *London, England* 523

Reply W. E. Mackenzie, D. S. Holmes, Ph.D., T. Webb, Ph.D., and J. R. Newton, M.D. *Edgbaston, Birmingham, England* 524

Information for authors on pages 27A, 28A, and 29A

Index to advertisers on page 60A

Bound volumes available to subscribers

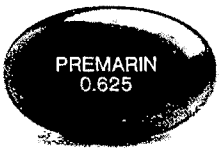
Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1987 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 156 (January-June) and Vol. 157 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by The C. V. Mosby Company for libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that 10¢ per page is paid directly to CCC, 29 Congress St., Salem, MA 01970; phone (617)744-3350. The U.S. Government retains a nonexclusive, royalty-free license in and to any copyright on articles authored by government employees. 0002-9378/87 $00.00 + .10.

If your patient is estrogen deficient, she needs proven results a transdermal can't deliver...

She needs



PREMARIN®

(Conjugated Estrogens Tablets)

Only oral estrogen provides

- Proven protection against osteoporosis
- Decreased total cholesterol; increased HDL cholesterol; lowered LDL cholesterol
- Easy cycling with progestin
- Unsurpassed long-term safety

PREMARIN®

Complete estrogen therapy

Please see following page for brief summary of prescribing information.

The appearance of PREMARIN Tablets is a trademark of Ayerst Laboratories.

BRIEF SUMMARY *(FOR FULL PRESCRIBING INFORMATION AND PATIENT INFORMATION, SEE PACKAGE CIRCULAR.)*

PREMARIN® Brand of conjugated estrogens tablets, USP

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent, case-controlled studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than one year. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade. The three case-controlled studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semi-annual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.

The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing, in later life, a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1,000 exposures. Furthermore, a high percentage of such exposed women (from 30% to 90%) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb-reduction defects. One case-controlled study estimated a 4.7-fold increased risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than 1 per 1,000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. If PREMARIN is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the advisability of pregnancy continuation.

DESCRIPTION: PREMARIN (conjugated estrogens tablets, USP) for oral administration contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters. Tablets are available in 0.3 mg, 0.9 mg, 1.25 mg, and 2.5 mg strengths of conjugated estrogens.

INDICATIONS AND USAGE: Moderate to severe *vasomotor* symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression without associated vasomotor symptoms and they should not be used to treat such conditions.) Osteoporosis (abnormally low bone mass). Atrophic vaginitis. Kraurosis vulvae. Female castration.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

Concomitant Progestin Use: The lowest effective dose appropriate for the specific indication should be utilized. Studies of the addition of a progestin for 7 or more days of a cycle of estrogen administration have reported a lowered incidence of endometrial hyperplasia. Morphological and biochemical studies of the endometrium suggest that 10 to 13 days of progestin are needed to provide maximal maturation of the endometrium and to eliminate any hyperplastic changes. Whether this will provide protection from endometrial carcinoma has not been clearly established. There are possible additional risks which may be associated with the inclusion of progestin in estrogen replacement regimens. (See PRECAUTIONS.) The choice of progestin and dosage may be important; product labeling should be reviewed to minimize possible adverse effects.

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 2. Known or suspected estrogen-dependent neoplasia. 3. Known or suspected pregnancy (see Boxed Warning). 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

WARNINGS: Estrogens have been reported to increase the risk of endometrial carcinoma (see Boxed Warning). However, a recent large, case-controlled study indicated no increase in risk of breast cancer in postmenopausal women. A recent study has reported a 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease in women receiving postmenopausal estrogens.

Adverse effects of oral contraceptives may be expected at the larger doses of estrogen used to treat prostatic or breast cancer or postpartum breast engorgement; it has been shown th there is an increased risk of thrombosis in men receiving estrogens for prostatic cancer ar women for postpartum breast engorgement. Users of oral contraceptives have an increased ris of diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarctio Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported oral contraceptive users. An increased risk of postsurgery thromboembolic complications ha also been reported in users of oral contraceptives. If feasible, estrogen should be discontinue at least 4 weeks before surgery of the type associated with an increased risk of thromboen bolism, or during periods of prolonged immobilization. Estrogens should not be used in persor with active thrombophlebitis, thromboembolic disorders, or in persons with a history of suc disorders in association with estrogen use. They should be used with caution in patients wi cerebral vascular or coronary artery disease. Large doses (5 mg conjugated estrogens per day comparable to those used to treat cancer of the prostate and breast have been shown t increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebiti When doses of this size are used, any of the thromboembolic and thrombotic adverse effec should be considered a clear risk.

Benign hepatic adenomas should be considered in estrogen users having abdominal pa and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has bee reported in women taking estrogen-containing oral contraceptives. Increased blood pressu may occur with use of estrogens in the menopause and blood pressure should be monitore with estrogen use. A worsening of glucose tolerance has been observed in patients c estrogen-containing oral contraceptives. For this reason, diabetic patients should be careful observed. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bor metastases.

PRECAUTIONS: Physical examination and a complete medical and family history should b taken prior to the initiation of any estrogen therapy with special reference to blood pressur breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a gener rule, estrogen should not be prescribed for longer than one year without another physic examination being performed. Conditions influenced by fluid retention, such as asthma, ep lepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patien may develop manifestations of excessive estrogenic stimulation, such as abnormal or exce sive uterine bleeding, mastodynia, etc. Prolonged administration of unopposed estroge therapy has been reported to increase the risk of endometrial hyperplasia in some patients. Or contraceptives appear to be associated with an increased incidence of mental depressio Patients with a history of depression should be carefully observed. Pre-existing uterin leiomyomata may increase in size during estrogen use. The pathologist should be advised estrogen therapy when relevant specimens are submitted. If jaundice develops in any patie receiving estrogen, the medication should be discontinued while the cause is investigate Estrogens should be used with care in patients with impaired liver function, renal insufficienc metabolic bone diseases associated with hypercalcemia, or in young patients in whom bon growth is not yet complete. If concomitant progestin therapy is used, potential risks may includ adverse effects on carbohydrate and lipid metabolism.

The following changes may be expected with larger doses of estrogen:

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increase norepinephrine-induced platelet aggregability.

c. Increased thyroic binding globulin (TBG) leading to increased circulating total thyroi hormone, as measured by PBI, T_4 by column, or T_4 by radioimmunoassay. Free T_3 resin uptak is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered.

d. Impaired glucose tolerance.

e. Decreased pregnanediol excretion.

f. Reduced response to metyrapone test.

g. Reduced serum folate concentration.

h. Increased serum triglyceride and phospholipid concentration.

As a general principle, the administration of any drug to nursing mothers should be done on when clearly necessary since mar drugs are excreted in human milk.

Long-term, continuous administra tion of natural and synthetic estr gens in certain animal species ir creases the frequency of carcinoma of the breast, cervix, vagina, and live However, in a recent, large case-cor trolled study of postmenopausa women there was no increase in ris of breast cancer with use of conju gated estrogens.

ADVERSE REACTIONS: The fo lowing have been reported with es trogenic therapy, including oral cor traceptives: breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea; pre menstrual-like syndrome; amenorrhea during and after treatment; increase in size of uterin fibromyomata; vaginal candidiasis, change in cervical erosion and in degree of cervical secre tion; cystitis-like syndrome; tenderness, enlargement, secretion (of breasts); nausea, vomiting abdominal cramps, bloating; cholestatic jaundice; chloasma or melasma which may persis when drug is discontinued; erythema multiforme; erythema nodosum; hemorrhagic eruption loss of scalp hair; hirsutism; steepening of corneal curvature; intolerance to contact lenses headache, migraine, dizziness, mental depression, chorea; increase or decrease in weigh reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea, and withdrawal bleeding may occur in females

DOSAGE AND ADMINISTRATION: 1. *Given cyclically for short-term use only.* For treatmer of moderate-to-severe *vasomotor* symptoms, atrophic vaginitis, or kraurosis vulvae associate with the menopause (0.3 mg to 1.25 mg or more daily).

The lowest dose that will control symptoms should be chosen and medication should b discontinued as promptly as possible.

Administration should be cyclic (eg, three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three- to six-month intervals

2. *Given cyclically:* Osteoporosis. Female castration.

Osteoporosis —0.625 mg daily. Administration should be cyclic (eg, three weeks on and on week off).

Female castration—1.25 mg daily, cyclically. Adjust upward or downward according to re sponse of the patient. For maintenance, adjust dosage to lowest level that will provide effectiv control.

Patients with an intact uterus should be monitored for signs of endometrial cancer an appropriate measures taken to rule out malignancy in the event of persistent or recurrin abnormal vaginal bleeding.

PREMARIN®

(Conjugated Estrogens Tablets)

Complete estrogen therapy

Ayerst® AYERST LABORATORIES
New York, NY 10017

Editors

JOHN I. BREWER, *Editor in Chief*
Sandra Fernandez, *Senior Editorial Assistant*

FREDERICK P. ZUSPAN, *Editor*
Meta M. Peterson, *Editorial Assistant*

E. J. QUILLIGAN, *Editor*
Rosalyn Vu, *Editorial Assistant*

ALBERT B. GERBIE, *Associate Editor*

Advisory committee on policy

William R. Anderson
Mason C. Andrews
E. Forrest Boyd
Amelia Cruz
Leo J. Dunn
A. Jerome Freeland
Morrie M. Gelfand
T. Terry Hayashi
Edgar O. Horger III
Hobart C. Hortman
Walter S. Keifer
Charles D. Kimball
Roger A. Lobo
Douglas J. Marchant
James H. Maxwell
Arthur M. McCausland
James A. Merrill
Frank C. Miller
Patrick T. Mohide
John C. Morrison
Richard Paul
Keith P. Russell
Ruth W. Schwartz
Joseph C. Scott, Jr.
Ruth Stockdale
Henry A. Thiede
Edward E. Wallach
Anne C. Wentz

Board of corresponding editors

Stuart Campbell, *England*
Luigi Carenza, *Italy*
Eliahu Sadovsky, *Israel*
Nils-Otto Sjöberg, *Sweden*
Masakuni Suzuki, *Japan*

Publisher
THE C. V. MOSBY COMPANY, 11830 Westline Industrial Drive,
St. Louis, Missouri 63146
Kay G. Goehler, *Senior Manuscript Editor*

American Journal of Obstetrics and Gynecology

Societies

in addition to those listed on the front cover the Journal is the official publication of the following societies

NEW YORK OBSTETRICAL SOCIETY
OBSTETRICAL SOCIETY OF PHILADELPHIA
BROOKLYN GYNECOLOGICAL SOCIETY
ST. LOUIS GYNECOLOGICAL SOCIETY
NEW ORLEANS GYNECOLOGICAL AND OBSTETRICAL SOCIETY
THE OBSTETRICAL AND GYNECOLOGICAL SOCIETY OF MARYLAND
CHICAGO GYNECOLOGICAL SOCIETY
CINCINNATI OBSTETRICAL AND GYNECOLOGICAL SOCIETY
WASHINGTON GYNECOLOGICAL SOCIETY
PITTSBURGH OBSTETRICAL AND GYNECOLOGICAL SOCIETY
BOSTON OBSTETRICAL SOCIETY
LOUISVILLE OBSTETRICAL AND GYNECOLOGICAL SOCIETY
SEATTLE GYNECOLOGICAL SOCIETY
ALABAMA ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
AKRON OBSTETRICAL AND GYNECOLOGICAL SOCIETY
KANSAS CITY GYNECOLOGICAL SOCIETY
CENTRAL NEW YORK ASSOCIATION OF GYNECOLOGISTS AND OBSTETRICIANS
NEW JERSEY OBSTETRICAL AND GYNECOLOGICAL SOCIETY
IOWA OBSTETRIC AND GYNECOLOGIC SOCIETY
THE TEXAS ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
OKLAHOMA CITY OBSTETRICAL AND GYNECOLOGICAL SOCIETY
MEMPHIS OBSTETRICAL AND GYNECOLOGICAL SOCIETY
UTAH OBSTETRICAL AND GYNECOLOGICAL SOCIETY
ROCHESTER OBSTETRICAL AND GYNECOLOGICAL SOCIETY
ARKANSAS OBSTETRICAL AND GYNECOLOGICAL SOCIETY
TENNESSEE STATE OBSTETRICAL AND GYNECOLOGICAL SOCIETY
NEW YORK GYNECOLOGICAL SOCIETY
PACIFIC NORTHWEST OBSTETRICAL AND GYNECOLOGICAL ASSOCIATION
BUFFALO OBSTETRICAL AND GYNECOLOGICAL SOCIETY
SAN FRANCISCO GYNECOLOGICAL SOCIETY
JACKSON GYNECIC SOCIETY
INDIANA OBSTETRICAL AND GYNECOLOGICAL SOCIETY
THE MINNESOTA OBSTETRICAL AND GYNECOLOGICAL SOCIETY

American Journal of Obstetrics and Gynecology

Copyright © 1987 by The C. V. Mosby Company

Information for Authors

Editors

John I. Brewer, *Editor in Chief*
710 North Fairbanks Court, Chicago, IL 60611

Sandra Fernandez, *Senior Editorial Assistant*
Tel.: (312) 908-5266

Frederick P. Zuspan, *Editor*
The Ohio State University, Fifth Floor, Means Hall, 1654 Upham Dr., Columbus, OH 43210

Meta M. Peterson, *Editorial Assistant*
Tel.: (614) 293-8798

E. J. Quilligan, *Editor*
University of California, Davis, School of Medicine, Department of Obstetrics and Gynecology, 1621 Alhambra Blvd., Suite 2500, Sacramento, CA 95816

Rosalyn Vu, *Editorial Assistant*
Tel.: (916) 453-5950

Albert B. Gerbie, *Associate Editor*
710 North Fairbanks Court, Chicago, IL 60611

Publisher
THE C. V. MOSBY COMPANY, 11830 Westline Industrial Drive, St. Louis, Missouri 63146

Kay G. Goehler, *Senior Manuscript Editor*
Tel.: (314) 872-8370

INFORMATION FOR AUTHORS

Editorial policies

The requirements for manuscripts submitted to the *American Journal of Obstetrics and Gynecology* conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors and published in *Annals of Internal Medicine* 1982;96(6):766-71. Certain requirements unique to our JOURNAL are provided in Information for Authors, published in each issue of the JOURNAL, and in more detail in the *Guide to Writing for the American Journal of Obstetrics and Gynecology.* The latter may be obtained from The C. V. Mosby Company or the Editors on request.

Manuscripts should be submitted to one of the three Editors as follows: (1) Dr. Brewer—all articles originating from the southeastern quadrant of the United States or Canada and those presented before one of the official sponsoring societies; (2) Dr. Zuspan—articles from the northeastern quadrant of the United States, Japan, Israel, Italy, and England, articles written for Clinical Opinion and Current Development, and Letters to the Editors; (3) Dr. Quilligan—articles from the north central states (including Ohio), states west of the Mississippi, Hawaii, Alaska, and abroad (except Japan, Israel, Italy, and England).

Most of the provisions of the Copyright Act of 1976 became effective on January 1, 1978. **Therefore, all manuscripts must be accompanied by the following written statement, signed by one author:** "The undersigned author transfers all copyright ownership of the manuscript [title of article] to The C. V. Mosby Company in the event the work is published. The undersigned author warrants that the article is original, is not under consideration by another journal, and has not been previously published. I sign for and accept responsibility for releasing this material on behalf of any and all co-authors." Authors will be consulted, when possible, regarding republication of their material.

When submitting a manuscript, an author should include, if applicable, a full statement about all submissions and prior reports in any medium that might be regarded as prior or duplicate publication of the same or similar work. Copies of such material should be included with the submitted manuscript. Articles previously published in another language will not be considered.

It is assumed by the Editors that articles emanating from a particular institution are submitted with the approval of the requisite authority. Articles dealing with human experimentation that require local institutional approval must have this approval prior to submission of the article for publication and it should be so indicated in the Methods section. Reports of experiments on animals must indicate which guidelines for the care and use of the animals were followed.

Statements and opinions expressed in articles and communications herein are those of the author(s) and not necessarily those of the Editor(s) or Publisher and the Editor(s) and Publisher disclaim any responsibility or liability for such material. Neither the Editor(s) nor the Publisher guarantees, warrants, or endorses any product or service advertised in this publication, nor do they guarantee any claim made by the manufacturer of such product or service.

General requirements for preparation of manuscripts

The original and two good-quality copies of the manu-

script and three glossy prints of illustrations are required.

Manuscripts must be typed double spaced on one side only on 22 by 28 cm (8 1/2 by 11 inch) white bond paper with 1 inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, abstract, body of text, references, legends, and tables.

Title page. The title page should contain in sequence the title (concise and suitable for indexing purposes); author line with first name, middle initial, and last name of all authors and their highest academic degree (both M.D. and Ph.D. are acceptable); city(ies), state(s) in which the study was conducted; divisional, or departmental, and institutional affiliations at the time the study was performed; acknowledgement of source(s) of support; presented line, if applicable; disclaimers, if any; name and address of author to whom requests for reprints should be addressed (if reprints will not be available, it should be so stated); and name and address of author responsible for correspondence concerning the manuscript if different from author to whom reprint requests are addressed. At the bottom of the title page supply a short title for the running head not exceeding 52 characters (including word spaces).

Abstract page and key words/phrases. On manuscript page two is the abstract, also typed double spaced with the required margins and headed by the title of the article and author(s) name(s). Abstracts for regular articles, Current Investigation, Clinical Opinion, and Current Development may not exceed 150 words. Abstracts for case reports and brief communications may not exceed 50 words. Below the abstract list 3 to 5 *key words* or short phrases for indexing purposes.

Text. Do not hesitate to write your manuscript in the first-person, active voice if it is more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations since the emphasis is on the "action" rather than the person performing the action.

Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors.* Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify Material and Methods, Results, and Comment. Authors may wish to summarize their findings in a short paragraph at the end of the Comment section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods section* describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results section* present the detailed findings. Include mentions of all tables and/or figures. Avoid duplication of text and supporting material. Emphasize only your important observations; do not compare your observations to those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment section* state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgements. Acknowledge only persons who have made substantive contributions to the study.

References. Limit of 16 references, except for case reports and brief communications, limited to 2, and Current Development, for which there is no limit. Number references consecutively in the order in which they are mentioned in the text. Use the format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals." Journal titles should conform to abbreviations used in *Cumulated Index Medicus.*

Illustrations and tables. A reasonable number of halftone and line illustrations will be reproduced without charge, but special arrangements must be made with the Editors for color reproduction. For color photographs, submit original transparencies and two sets of unmounted prints on glossy paper with good color balance. For black and white illustrations, submit three sets of 3 by 4 inch (minimum) to 5 by 7 inch (maximum) unmounted glossy photographic prints. Figures must be cited consecutively in the text in Arabic numerals and identified thusly on the back of the print (gummed label with author(s) name(s), title of article, number, and top marked clearly). Do not use paper clips. Legends must accompany all figures; type double spaced on a single sheet of paper if possible and number with manuscript in sequence after the references. Titles should be included in the legend, *not* on the print.

All lettering must be done with commercially available paste-on letters (or numbers) or by a professional; typed or freehand lettering is not acceptable. All lettering must be in proportion to the drawing, graph, or photograph. Original drawings, appropriately done in black India ink, roentgenograms, and other material must be submitted as glossy photographic prints with good black and white contrast. Consistency in size within the article is strongly preferred. Any special instructions regarding sizing should be clearly noted. Illustrations will be returned only on the author's request.

Tables should be typed on separate sheets of paper and included at the end of the text, one to a page. They should be numbered in Roman numerals. Each table must be cited

Estimating length of manuscript

The length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A 4200 word text can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7 page article in the JOURNAL. The 2000 words of text alone make approximately 8 pages of manuscript typed double spaced with the required 1 inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 typed words in manuscript. Thus if a greater number of illustrations and tables are used, the length of the text should be adjusted accordingly.

in sequence at an appropriate point in the text. Captions should be brief yet indicate clearly the purpose or content of each table and each column precisely defined by headings. Abbreviations and special designations should be explained in a footnote to the table. Special arrangements must be made with the Editors for elaborate tables because of space limitations.

Illustrations and tables should supplement, not duplicate, the text; presentation in either one or the other will suffice.

Permissions and photographic consent. Previously published tables and figures must be correctly referenced and written permission from the copyright holder included with the manuscript. Photographs of identifiable persons must be accompanied by signed releases or else all recognizable features masked.

Requirements for special sections

Case reports and brief clinical and basic science communications. Limit of 700 words, 2 references. Include abstract of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures are used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

Current Investigation. Same requirements as for a regular article.

Clinical Opinion. Limit of 3000 words, 16 references. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Current Development. Limit of 6000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Correspondence. Two types of correspondence will be considered for publication. (1) A Letter to the Editors commenting on an article that has appeared in the JOURNAL should be brief and directly related to the published article. The editorial staff reserves the right to shorten letters if necessary and to make minor editorial alterations without reference to the writer. Letters may be published together with a reply from the original author. As space for letters is limited, only a selection of letters submitted may be published. (2) A brief case presentation or a short report of a pertinent observation in the form of a Letter to the Editors will be considered for publication. All letters should be typed double spaced. Letters should be sent to Dr. Zuspan.

Announcements. Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

Books. Books received will be listed in the JOURNAL. They should be sent to Dr. Gerbie. No books will be returned.

Reprints. Reprints of articles must be obtained from the author. The corresponding author will receive a price schedule and order form at the time of publication. Reprints in quantities must be ordered from the Publisher with the author's consent.

Business communications. Communications of a business nature and all advertising communications should be addressed to: Journal Publisher, The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, or call Journal Advertising Production Manager (314) 872-8370.

Checklist

—Letter of submission
—Copyright transfer letter
—Original and two xerographic copies of manuscript
—Title page
  —Title of article
  —Full name(s) and affiliations of author(s)
  —Author to whom correspondence is to be sent
  —Reprint request line or line stating reprints not available
  —Short title
—Abstract (double spaced), 3 to 5 key words/phrases
—Article proper (double spaced)
—References (double spaced), on a separate sheet
—Legends (double spaced), on a separate sheet
—Tables (double spaced), each on a separate sheet
—Illustrations, properly labeled (three copies of glossy prints)
—Permission to reproduce published material

The competition is
significant gaps in

fact:
Excellent Clinical Results in Intra-abdominal Infections* Compared with Mefoxin® (cefoxitin/MSD)

Ref.	Investigator	Year	Therapy	Satisfactory Response (No. of cases)
1	Lou et al.	1985	Cefizox® (ceftizoxime)	100% (48/48)
			cefoxitin	98% (60/61)

Clinical response was based upon subjective evaluation by the investigator; no differentiation between cure and improvement was made.

fact:
Enteric Microflora Profile Superior to Mefoxin®

Enteric Pathogens†		Cefizox®	Mefoxin®
Refs.	Organisms (No. of strains)	MIC_{90} (mcg./ml.)	MIC_{90} (mcg./ml.)
2	*Escherichia coli (40)*	0.04	6.3
2	*Klebsiella pneumoniae (32)*	0.05	25
2	*Enterobacter aerogenes (16)*	0.12	>400
2	*Proteus* (indole+) *(26)*	6.3	12.5
2	*Serratia marcescens (32)*	12.5	>100
3	*Providencia rettgeri*	0.06	64
4	*Bacteroides fragilis (55)*	8	16

*Due to susceptible strains of indicated organisms.
†*In vitro* activity does not necessarily imply *in vivo* effectiveness.

1. Lou, M.A., et al.: Comparative evaluation of ceftizoxime and cefoxitin in the treatment of intraabdominal infections, 14th Internat. Congr. Chemother., June 1985, Kyoto, Japan, pp. 2378-2379.
2. Fu, K.P., and Neu, H.C.: Antibacterial activity of ceftizoxime, a β-lactamase-stable cephalosporin, *Antimicrob. Agents Chemother.* 17:583-590, Apr. 1980.
3. Thornsberry, C.: Review of in vitro activity of third-generation cephalosporins and other newer beta-lactam antibiotics against clinically important bacteria, *Am. J. Med.* 79: (Suppl. A) 14-20, Aug. 1985.
4. Aldridge, K.E., et al.: Comparison of the activities of penicillin G and new β-lactam antibiotics against clinical isolates of *Bacteroides* species, *Antimicrob. Agents Chemother.* 26:410-413, Sept. 1984.

fact:
Lower Cost of Therapy than Mefoxin®

Average Hospital Costs of Therapy for Severe Infections	Cefizox®	Mefoxi
Cost per Unit	$9.13/g*	$6.82/
Recommended Dosage for Severe Infections	2g. q8-12h	2g. q6-
Cost per Day†	$55 to $37	$55 to $
Preparation and Administration Cost per Day‡	$9 to $6	$12 to $
Total Cost of Therapy per Day	$64 to $43	$67 to $
Total Cost of Therapy per 7 Days of Treatment	$448 to $301	$469 to $
Savings with CEFIZOX®	**$21 to $49**	

* Price quoted = wholesaler's price + 5%.
† Rounded to the nearest dollar.
‡ Estimated @ $3/dose.

- **Offers b.i.d. dosing for all but life-threatening infections**
- **Outstanding mixed aerobic/anaerobic coverage**
- **Excellent safety profile**
- **Lower cost of therapy than Mefoxin®**

right! Why risk coverage?

the facts *actually* favor

Cefizox® IM IV

brand of sterile

ceftizoxime sodium



ase see brief summary of prescribing
ormation on last page of this advertisement.

Cefizox®

brand of

sterile ceftizoxime sodium and ceftizoxime sodium injection

Before prescribing, see complete prescribing information in SK&F literature or *PDR.* The following is a brief summary.

INDICATIONS

'Cefizox' is indicated in the treatment of infections due to susceptible strains of the microorganisms listed below:

LOWER RESPIRATORY TRACT INFECTIONS caused by *Streptococcus* sp. including *S. pneumoniae* (formerly *D. pneumoniae*), but excluding enterococci; *Klebsiella* sp.; *Proteus mirabilis; Escherichia coli; Haemophilus influenzae* including ampicillin-resistant strains; *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Serratia* sp.; *Enterobacter* sp.; and *Bacteroides* sp.

URINARY TRACT INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Escherichia coli; Pseudomonas* sp. including *Ps. aeruginosa; Proteus mirabilis; P. vulgaris; Providencia rettgeri* (formerly *Proteus rettgeri*) and *Morganella morganii* (formerly *Proteus morganii*); *Klebsiella* sp.; *Serratia* sp. including *S. marcescens; Enterobacter* sp.

GONORRHEA. Uncomplicated cervical and urethral gonorrhea caused by *Neisseria gonorrhoeae.*

INTRA-ABDOMINAL INFECTIONS caused by *Escherichia coli; Staphylococcus epidermidis; Streptococcus* sp. (excluding enterococci); *Enterobacter* sp.; *Klebsiella* sp.; *Bacteroides* sp. including *B. fragilis;* and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

SEPTICEMIA caused by *Streptococcus* sp. including *S. pneumoniae* (formerly *D. pneumoniae*), but excluding enterococci; *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Escherichia coli; Bacteroides* sp. including *B. fragilis; Klebsiella* sp.; and *Serratia* sp.

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Staphylococcus epidermidis; Escherichia coli; Klebsiella* sp.; *Streptococcus* sp. including *Streptococcus pyogenes* (group A beta-hemolytic), but excluding enterococci; *Proteus mirabilis; Serratia* sp.; *Enterobacter* sp.; *Bacteroides* sp. including *B. fragilis;* and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

BONE AND JOINT INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Streptococcus* sp. (excluding enterococci); *Proteus mirabilis; Bacteroides* sp.; and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

MENINGITIS caused by *Haemophilus influenzae.* 'Cefizox' has also been used successfully in the treatment of a limited number of pediatric and adult cases of meningitis caused by *Streptococcus pneumoniae.*

'Cefizox' has been effective in the treatment of seriously ill, compromised patients, including those who were debilitated, immunosuppressed or neutropenic.

Many infections caused by aerobic gram-negative organisms and by mixtures of organisms resistant to other cephalosporins, aminoglycosides, or penicillins respond to treatment with 'Cefizox'.

Because of the serious nature of some urinary tract infections due to *Ps. aeruginosa* and because many strains of *Pseudomonas* species are only moderately susceptible to 'Cefizox', higher dosage is recommended. Other therapy should be instituted if the response is not prompt.

Susceptibility studies on specimens obtained prior to therapy should be used to determine the response of causative organisms to 'Cefizox'. Therapy with 'Cefizox' may be initiated pending results of the studies; however, treatment should be adjusted according to study findings. In serious infections 'Cefizox' has been used concomitantly with aminoglycosides (see Precautions). Before using 'Cefizox' concomitantly with other antibiotics, the prescribing information for those agents should be reviewed for contraindications, warnings, precautions and adverse reactions. Renal function should be carefully monitored.

CONTRAINDICATIONS

'Cefizox' is contraindicated in persons who have shown hypersensitivity to cephalosporin antibiotics.

WARNINGS

BEFORE THERAPY WITH 'CEFIZOX' IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE WHETHER THE PATIENT HAS HAD PREVIOUS HYPERSENSITIVITY REACTIONS TO CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. THIS PRODUCT SHOULD BE GIVEN CAUTIOUSLY TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients w developed diarrhea in association with antibiotic use. Treatment with broad-spectrum antibioti alters normal flora of the colon and may permit overgrowth of *Clostridia.* Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin *in vitro.* Mild cases o colitis may respond to drug discontinuance alone. Moderate to severe cases should be manag with fluid, electrolyte and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance or when it is severe, oral vancomycin is the treatment of choice for an biotic-associated pseudomembranous colitis produced by *C. difficile.* Other causes of colitis should also be considered.

PRECAUTIONS

General: As with all broad-spectrum antibiotics, 'Cefizox' should be prescribed with caution i individuals with a history of gastrointestinal disease, particularly colitis.

Although 'Cefizox' has not been shown to produce an alteration in renal function, renal status should be evaluated, especially in seriously ill patients receiving maximum dose therapy. As with any antibiotic, prolonged use may result in overgrowth of nonsusceptible organisms. Car ful observation is essential; appropriate measures should be taken if superinfection occurs.

Drug Interactions: Although the occurrence has not been reported with 'Cefizox,' nephrotoxicity has been reported following concomitant administration of other cephalosporins and aminoglycosides.

Pregnancy: (Category B.) Reproduction studies have been performed in rats and rabbits and have revealed no evidence of impaired fertility or harm to the fetus due to 'Cefizox'. There ar however, no adequate and well-controlled studies in pregnant women. Because animal reprodu tion studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Labor and Delivery: Safety of 'Cefizox' use during labor and delivery has not been established

Nursing Mothers: 'Ceftizox' is excreted in human milk in low concentrations. Caution should l exercised when 'Cefizox' is administered to a nursing woman.

Infants and Children: Safety and efficacy in infants from birth to six months of age have not bee established. In children six months of age and older, treatment with 'Cefizox' has been associat with transient elevated levels of eosinophils, SGOT, SGPT and CPK (creatine phosphokinase). The CPK elevation may be related to I.M. administration.

ADVERSE REACTIONS

Ceftizoxime is generally well tolerated; the *most* frequent adverse reactions (*greater than* 1%, b less than 5%) are: *Hypersensitivity*—Rash, pruritus, fever. *Hepatic*—Transient elevation in SGC SGPT and alkaline phosphatase. *Hematologic*—Transient eosinophilia, thrombocytosis. Some individuals have developed a positive Coombs test. *Local*—Injection site—Burning, cellulitis, phlebitis with I.V. administration, pain, induration, tenderness, paresthesia.

Less frequent adverse reactions (*less than* 1%) are: *Hypersensitivity*—Numbness has been reported rarely. *Hepatic*—Elevation of bilirubin has been reported rarely. *Renal*—Transient elev tions of BUN and creatinine have been occasionally observed with ceftizoxime. *Hematologic*-Anemia, leukopenia, neutropenia and thrombocytopenia have been reported rarely. *Genitourinary*—Vaginitis has rarely occurred. *Gastrointestinal*—Diarrhea; nausea and vomitir have been reported occasionally. Symptoms of pseudomembranous colitis can appear during or after antibiotic treatment. (See Warnings.)

HOW SUPPLIED

Cefizox (sterile ceftizoxime sodium, FSK) is supplied in vials equivalent to 1 gram or 2 grams ceftizoxime; in "Piggyback" Vials for I.V. admixture equivalent to 1 gram or 2 grams of ceftizoxin

Cefizox (ceftizoxime sodium injection, FSK) as a frozen, sterile, nonpyrogenic solution in plastic containers—supplied in 50 ml. single-dose containers equivalent to 1 gram or 2 grams ceftizoxime, in 5% Dextrose (D5W). Do not store above -20°C.

'Cefizox' supplied as a frozen, sterile, nonpyrogenic solution in plastic containers is manufacture for Fujisawa SmithKline Corporation by Travenol Laboratories, Inc., Deerfield, IL 60015.

BRS—CF:L14

© Smith Kline &French Laboratories, 1987
Division of SmithKline Beckman Corporatic

FSK Manufactured for
Fujisawa SmithKline Corporation
Distributed by
SK&F Laboratories
Division of SmithKline Beckman Corp.

American Journal of Obstetrics and Gynecology

Founded in 1920

volume 156 *number* 2 FEBRUARY 1987

TRANSACTIONS OF THE FORTY-SECOND ANNUAL MEETING OF THE SOCIETY OF OBSTETRICIANS AND GYNAECOLOGISTS OF CANADA

Psychological characteristics of in vitro fertilization participants

M. T. Hearn, Ph.D., A. A. Yuzpe, M.D., S. E. Brown, M.D., and R. F. Casper, M.D.

London, Ontario, Canada

Psychological characteristics of couples attending an in vitro fertilization clinic were examined. Participants completed measures of personality, family environment, life quality, coping, and emotional status. Couples seeking in vitro fertilization exhibited specific personality traits and cohesive, supportive relationships. Unexpectedly, they reported good quality of life and freedom from anxiety and depression. (AM J OBSTET GYNECOL 1987;156:269-74.)

Key words: In vitro fertilization, psychological characteristics of participants

Since the first successful intrauterine pregnancy in 1979, hundreds of pregnancies have been reported worldwide as a result of in vitro fertilization and embryo transfer procedures. Most of the literature on this procedure is focused on the medical aspects of the in vitro fertilization procedure, which has moved rapidly from an experimental technique to a successful medical treatment for some forms of infertility. Some concern about the psychological aspects of in vitro fertilization has accompanied the development of in vitro fertilization treatment programs.[1, 2] Some authors[3, 4] have emphasized the need for psychological screening to single out those applicants for whom an in vitro fertilization attempt might be emotionally contraindicated. Some[1-5] have emphasized the need for psychological support, and others[5, 6] have investigated the degree of dysfunctional emotional distress shown by in vitro fertilization participants. Few studies have investigated the psychological characteristics of couples seeking in vitro fertilization, and thus far there is little known about the psychological and emotional states of couples entering in vitro fertilization programs. This lack of information makes it difficult to plan adequate support systems for couples entering in vitro fertilization programs.

This article describes the psychological characteristics of couples entering an in vitro fertilization treatment program in a university teaching hospital and discusses briefly the type of support procedures that would complement participants' needs. These pretreatment assessment data also represent a baseline for future study of couples who complete a successful pregnancy as a result of an in vitro fertilization attempt in comparison with couples who are unsuccessful.

Material and methods

Criteria. Couples entering the in vitro fertilization treatment program were required to meet the following criteria: (1) a stable marital relationship; (2) age of 40 years or under; (3) evidence of tubal disease, which might include failed tubal surgery, surgical repair with <10% chance of pregnancy, or absent fallopian tubes; (4) sperm count in the husband within the normal fertile range; and (5) exhaustion of other attempts to restore fertility.

Sample selection. The sample comprised 300 couples who were consecutive admissions to the in vitro

From the Department of Psychology and the Department of Gynaecology, University Hospital.

Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.

Reprint requests: Margaret T. Hearn, Ph.D., Department of Psychology, University Hospital, P. O. Box 5339, Station A, London, Ontario, Canada N6A 5A5.

Table I. Comparison of the in vitro fertilization group with the normative data for the family environment scale

Scale	*In vitro fertilization group*		*Normative group*		t
	Mean	*SD*	*Mean*	*SD*	
Cohesion	8.02	1.42	6.36	1.86	12.06*
Expressiveness	6.41	1.77	5.43	1.49	7.20*
Conflict	2.10	1.79	4.65	2.05	15.39*
Independence	6.75	1.48	6.67	1.23	0.71
Achievement orientation	5.37	1.63	5.64	1.66	1.97
Intellectual-cultural orientation	5.40	2.15	6.15	1.98	4.35*
Active-recreational orientation	5.85	2.12	6.19	1.66	2.15
Moral-religious emphasis	5.15	2.08	4.55	2.15	3.41*
Organization	6.76	1.77	5.27	2.03	9.40*
Control	3.70	1.93	4.80	1.84	6.98*

*p < 0.001.

Table II. Comparison of the in vitro fertilization group women with the normative data for the Personality Research Form-E

Scale	*In vitro fertilization group*		*Normative group*		t
	Mean	*SD*	*Mean*	*SD*	
Abasement	6.96	2.63	7.66	3.01	2.39
Achievement	9.91	2.56	10.00	3.41	0.29
Affiliation	9.75	3.26	8.93	4.03	2.16
Aggression	6.32	2.75	6.91	3.59	1.78
Autonomy	4.55	2.56	7.11	3.23	8.50*
Change	7.44	2.53	9.87	3.09	8.34*
Cognitive structure	10.28	2.59	8.71	3.52	4.92*
Defendence	5.99	2.72	6.04	3.48	0.16
Dominance	6.70	3.65	7.60	4.40	2.17
Endurance	10.52	2.75	10.19	3.33	1.04
Exhibition	6.31	3.67	7.24	4.76	2.12
Harm avoidance	12.83	2.78	9.49	4.43	8.75*
Impulsivity	4.76	3.23	6.53	4.04	4.68*
Nurturance	11.9	2.51	10.90	3.42	3.38†
Order	11.12	3.92	8.15	4.52	6.80*
Play	7.25	2.70	8.95	3.02	5.74*
Sentience	9.26	3.10	10.76	2.75	4.95*
Social recognition	7.42	3.04	8.22	3.68	2.28
Succorance	8.80	3.14	8.70	3.70	0.27
Understanding	8.11	3.33	9.70	3.49	4.51*
Infrequency	0.52	1.40	0.37	0.78	1.28
Desirability	12.78	2.30	10.97	2.53	7.26*

*p < 0.01.
†p < 0.05.

fertilization treatment program during an 18-month period between January 1984 and July 1985.

Measures. The following psychological self-report measures were used.

The Family Environment Scale[7] measures the social-environmental characteristics of families, and there are 10 subscales. Scores on the subscales indicate an individual's perception of the conjugal or nuclear family environment.

The Personality Research Form-E[8] measures personality characteristics of individuals, and there are 20 content scales and two validity scales. Scores on the content scales indicate an individual's style of normal functioning on traits such as autonomy, nurturance, and endurance.

The Quality of Life Questionnaire[9] assesses an individual's functional behavior in response to several ecologic domains. Major domains assessed include general well-being, interpersonal relations, organizational activity, occupational activity, and leisure-recreational activity.

The Life Satisfaction Questionnaire[10] yields a score

Table III. Comparison of the in vitro fertilization group men with the normative data for the Personality Research Form-E

Scale	*In vitro fertilization group*		*Normative group*		t
	Mean	*SD*	*Mean*	*SD*	
Abasement	6.79	2.69	7.78	2.76	3.52*
Achievement	11.16	2.67	10.98	3.12	0.60
Affiliation	9.57	3.32	8.33	3.70	3.40*
Aggression	6.91	3.07	7.35	3.36	1.32
Autonomy	5.96	2.77	9.54	3.59	10.76†
Change	7.72	2.76	9.49	2.86	6.08†
Cognitive structure	10.22	2.84	8.64	3.38	4.87†
Defendence	6.12	3.07	5.75	3.53	1.08
Dominance	9.74	3.80	10.19	4.31	1.06
Endurance	11.20	2.74	10.92	3.11	0.91
Exhibition	7.21	3.78	7.52	4.16	0.76
Harm avoidance	9.63	3.59	7.41	4.03	5.60†
Impulsivity	4.20	2.98	5.46	3.66	3.65†
Nurturance	10.33	2.90	8.90	3.67	4.17†
Order	9.50	4.14	7.82	4.73	3.65†
Play	6.95	2.81	8.18	3.57	3.70†
Sentience	7.80	3.15	9.28	3.63	4.17†
Social recognition	7.48	2.99	7.52	3.75	0.13
Succorance	6.64	3.18	5.64	3.73	2.79
Understanding	7.75	3.46	10.25	3.26	7.18†
Infrequency	0.45	1.21	0.48	0.72	0.34
Desirability	12.85	2.14	10.78	3.00	7.65†

*$p < 0.05$.
†$p < 0.01$.

on a one-item rating scale measuring satisfaction with life in general.

The Life Appraisal Inventory[10] results in an affect balance score representing the degree of affect, along a positive-negative continuum, associated with an individual's life.

The Inventory of Socially Supportive Behaviors[11] measures the frequency of socially supportive contacts.

The Ways of Coping Checklist (Revised)[12] provides information concerning the strategies an individual uses to cope with a specific stressful life event.

The State-Trait Anxiety Inventory[13] measures two distinct anxiety concepts. The state anxiety scale yields a measure of how a subject feels at a particular moment in time, as opposed to the trait anxiety scale, which measures how a subject feels in general.

The Beck Depression Inventory[14] measures an individual's current level of depression.

Demographic information and reasons for wanting a child were also solicited.

Procedure. On meeting the medical criteria and approximately 3 months before the first visit to the in vitro fertilization clinic, prospective couples were sent two packages of the above inventories. In an explanatory letter, husband and wife were requested to complete a single package of inventories independently and to return these to the program. The questionnaires were scored and used as both a data base and a source of information for the clinical interview. On assessment day, couples were engaged in a structured interview designed to assess their attitudes to and preparation for the in vitro fertilization program. Interpretation of the assessment information was undertaken, concerns about psychological responses to the in vitro fertilization process were discussed, and, where appropriate, improved modes of coping were considered. Another use of the interview was to substantiate the questionnaire data. As there was high concordance for all participants between questionnaire and interview responses, no data were excluded because they were questionable.

Results

Characteristics of the sample. The mean age of the group was 32 years and the socioeconomic status of the group was 5.5 on the Hollingshead scale of social status (Hollingshead AB. Four factor index of social status. New Haven: Department of Sociology, Yale University, 1975, unpublished manuscript.). A mean income of $40,000 was reported for couples. Thirty-five percent of the group reported education beyond high school, of which 15% graduated from a university. Overall, the mean educational level of the group was completion of grade 12. Twenty-six percent of the group reported themselves as Protestant in their religious preference, 26% Catholic, 1% Jewish, and 49% other or none.

Table IV. Comparison of the in vitro fertilization group with the normative data for selected scales of the Quality of Life Questionnaire

Scale	*In vitro fertilization group*		*Normative group*		t
	Mean	*SD*	*Mean*	*SD*	
Material well-being	10.00	1.54	9.55	1.93	2.90
Personal growth	9.60	2.03	8.46	2.40	5.78*
Marital relations	10.44	1.80	8.64	2.83	8.55*
Parent-child relations	8.75	2.20	7.94	2.56	2.78
Extended family relations	9.33	2.50	7.99	2.94	5.53*
Extrafamilial relations	8.74	4.08	7.71	2.50	3.43*
Job characteristics	8.77	2.32	7.60	2.99	4.51*
Occupational relations	8.54	2.77	7.89	2.96	2.18
Job satisfiers	7.97	2.56	6.62	2.97	4.81*
Creative-aesthetic behavior	6.33	2.44	6.11	2.54	1.00
Sports activity	5.90	2.90	5.90	3.25	0.00
Vacation behavior	9.17	2.07	8.35	3.37	3.30†
Social desirability	8.30	2.10	7.65	2.37	3.26†

*$p < 0.01$.
†$p < 0.05$.

Table V. Means and standard deviations for the in vitro fertilization group on coping and social support measures

Scale	*Mean*	*SD*
Ways of coping		
Problem-focused coping	13.08	6.65
Wishful thinking	5.22	3.42
Distancing	4.85	3.04
Seeking social support	8.69	4.58
Emphasizing the positive	5.05	2.87
Self-blame	1.34	1.68
Tension reduction	1.52	1.55
Self-isolation	1.98	1.84
Inventory of socially supportive behaviors	79.80	18.52

Twenty percent of the group had been previously married and divorced, and 1% was widowed. Couples reported a mean of 0.4 children for their current relationship and 0.3 for a previous relationship. This compares with a national average of 1.3 children per couple. In order of priority, couples endorsed the following as important reasons for wanting a child: strong desire, 82%; belief in their own ability to provide good parenting, 64%; desire of spouse for a child, 58%; and life incomplete without children, 33%.

Family environment. Compared with the normative sample for the Family Environment Scale, the in vitro fertilization couples indicated significantly greater organization, cohesion, expressiveness, and a stronger moral-religous emphasis in their relationship (see Table I). In contrast they reported significantly lower levels of conflict, control, and intellectual-cultural orientation.

Personality. Women and men in the in vitro fertilization group, in comparison with the normative sample of the Personality Research Form-E, reported a significantly higher need for cognitive structure, harm avoidance, nurturance, and order (see Tables II and III). Conversely they indicated a significantly lower need for autonomy, change, play, sentience, and understanding. Both women and men responded to the Personality Research Form-E in a more socially desirable manner.

Quality of life. Couples in the in vitro fertilization group did not differ from the North American normative sample in overall life satisfaction, general affect, or overall well-being. On the more specific Quality of Life Questionnaire they reported higher levels of personal growth, marital relations, extended family relations, extrafamilial relations, job characteristics, job satisfiers, and vacation behavior than the normative group (see Table IV). Again, increased social desirability bias in responding was observed.

Coping and social support. Comparable normative data for the coping and social support measures were not available. The means and standard deviations for these measures are shown in Table V. That coping and social support activities are effective is demonstrated by the fact that men and women in the in vitro fertilization group do not differ from normal samples with respect to depression (see Table VI). With respect to anxiety the women do not differ from the normative sample, and the men are significantly less trait and state anxious than the normal sample.

Comment

The findings of this investigation relate to an organic sample only; hence they cannot be generalized to all in vitro fertilization candidates. As well, respondents

Table VI. Comparison of women and men in the in vitro fertilization group with the normative data for the State-Trait Anxiety Inventory

Scale	*In vitro fertilization group*		*Normative group*		t
	Mean	*SD*	*Mean*	*SD*	
Women					
State anxiety	33.41	10.42	35.20	10.61	1.61
Trait anxiety	35.49	8.24	34.79	4.22	0.76
Beck Depression Inventory	4.40	5.69	5.82	5.27	1.84
Men					
State anxiety	31.15	8.41	35.72	10.40	5.23*
Trait anxiety	32.87	8.17	34.89	9.19	2.48†
Beck Depression Inventory	3.26	6.37	3.97	3.94	0.86

Normative data for the State-Trait Anxiety Inventory are from Spielberger[13] and for the Beck Depression Inventory are from Lips.[16]

*$p < 0.01$.

†$p < 0.05$.

evidenced some bias toward socially acceptable responding, possibly because of their concern that they be psychologically acceptable for an in vitro fertilization program. A further limitation in the interpretation of the current findings is the lack of a relevant control group. However, definition of a control group for an in vitro fertilization population is difficult in that few other stressful life events: (1) continue for the length of time encompassed by an infertility problem, (2) have a comparable probability of success, and (3) involve an invasive procedure. Finally the data for coping and social support are included for descriptive purposes only, as these instruments lack normative data at this time. Despite these limitations, the investigation of psychological characteristics of in vitro fertilization couples reveals information about the functioning of these couples, and this can be utilized in developing support programs to complement their emotional needs.

In this study couples presenting for in vitro fertilization–embryo transfer treatment exhibited closer marital relationships, a more conservative approach to life, a higher quality of life, and emotional adjustment and coping comparable to that of a normal population. Many of these couples have attempted resolution of their infertility during a period of many years. Hence it is possible that the characteristics and coping strategies they exhibit result from their experiences in seeking resolution of their infertility.

The marital relationship of these couples is one of openness, support, and warmth, with clear lines of marital organization and little concern about control. Although considerable attention has been given to the negative effects of infertility,[15] this has not been accompanied by investigation of the positive aspects of those relationships that survive infertility. It is conceivable that as a result of confronting their infertility couples develop a fuller, richer relationship that can be utilized as a source of strength during an in vitro fertilization treatment.

Couples entering in vitro fertilization programs have been investigated for psychopathologic conditions and there is evidence that these exist.[5, 6] However, the majority of couples entering an in vitro fertilization program will present with normal personality functioning. While some programs may wish to screen for this, the low level of psychopathologic findings in the current sample (1%) suggests that concentration on personality styles and coping mechanisms rather than psychopathologic conditions may prove more profitable in assisting couples to cope with stress as they proceed through an in vitro fertilization program. In the current study, couples undergoing in vitro fertilization presented as serious individuals with high needs for orderly, predictable, threat-free environments that offer them opportunity for support.

Since many aspects of the in vitro fertilization attempt are unpredictable (medication response, egg retrieval, fertilization, and outcome), it is likely that many of these couples will find themselves in an environment that is not complementary to their personality needs. To rectify this, in vitro fertilization programs could provide couples with accurate and adequate information about what might occur at any and every stage of the in vitro fertilization process. The degree to which a program provides structure through information meetings, audio-video tapes, literature, and availability of well-trained staff may well determine the degree of stress that couples experience as they proceed through an in vitro fertilization program.

The findings from the Quality of Life Inventory indicate that, despite the lack of fulfillment of their desire for children, infertile couples develop an above-average quality of life. Possibly the time and expense that would otherwise be expended on children are directed

toward activities that are personally rewarding and meaningful.

Normal depression and anxiety levels were unexpected in this population, in view of the distress that has been attributed to infertility[15] and the stress that is associated with the in vitro fertilization treatment. The participants in this study showed low levels of depression that were comparable to those of the nonpregnant women and nonexpectant men in the Canadian sample studied by Lips.[16] Given the anticipation of a potential solution to their infertility problem, a lack of depression in this population is reasonable before entry into the program. When compared with participants in the study of Dennerstein and Morse,[17] women in the present organic sample showed lower levels of both trait and state anxiety than their organic and idiopathic groups. In the current study, the sample size and participation rate suggest that it is appropriate to conclude that 3 months before entry into the program trait and state anxiety are within normal limits for women seeking in vitro fertilization for organic reasons. In the study by Dennerstein and Morse,[17] high levels of anxiety were reported for the organic and idiopathic groups. However, it is unclear whether these levels of anxiety differed significantly from those of the normative sample. Further, their small sample size and method of sampling may have resulted in atypical groups. This possibility is partially supported by the fact that their female sample was characterized by a long history of hospitalizations extending back to childhood and a 26% rate of previous psychiatric involvement. These were not the characteristics of the current sample. It is important, however, to note that couples completed the questionnaires for the current study 3 months before their entry into the program. It is possible that anxiety levels might have increased by the time of the actual attempt.

The use of problem solving as a coping strategy has been associated with normal levels of emotional responsivity,[12] and certainly problem solving was endorsed as a prime coping strategy by in vitro fertilization participants in this study. However, recent research on coping and emotions indicates the need to view these variables as process rather than static variables.[12] Thus further research is needed to evaluate both coping strategies and emotional levels of in vitro fertilization participants as they proceed through in vitro fertilization attempts.

Couples who enter in vitro fertilization programs are more likely to face negative outcomes than positive ones and therefore will be required to cope with the emotional distress associated with a negative outcome. On the basis of experience with the current program, identification of psychological characteristics of in vitro fertilization participants permits the development of appropriate programming and staff support. As well, knowledge of psychological characteristics of this population may result in a better understanding of why some couples undergoing in vitro fertilization proceed to a successful pregnnacy but others do not.

REFERENCES

1. Committee to consider the social, ethical and legal issues arising from in vitro fertilization: interim report. Victoria, Australia: Government Printer 1982:22.
2. Muller HJ. Human in vitro fertilization and embryo transfer: expectations and concerns. Experientia 1985;41:1515.
3. Stauber M, Maasser V, Spielmann H, Dineer C. Psychosomatic counselling of couples involved in an in vitro fertilization (IVF)-embryo transfer (ET) program, Experientia 1985;41:1514.
4. Campagnoli C, Di Gregorio R, Arisio R, Fessia L. Patient selection for in vitro fertilization (IVF) and embryo transfer (ET). Experientia 1985;41:1491.
5. Freeman EW, Boxer AS, Rickels K, Tureck R, Mastroianni L. Psychological evaluation and support in a program of in vitro fertilization and embryo transfer. Fertil Steril 1985;43:48.
6. Garner CH, Kelly M, Arnold ES. Psychological profile of IVF patients. Fertil Steril 1984;41:575.
7. Moos RH, Moos BS. Family environment scale manual. Palo Alto: Consulting Psychologists Press, 1981.
8. Jackson DN. Personality research form manual. Port Huron: Research Psychologists Press, 1984.
9. Evans DR, Burns JE, Robinson WE, Garrett OJ. The Quality of Life Questionnaire: a multidimensional measure. Am J Community Psychol 1985;13:385.
10. Campbell A, Converse PE, Rodgers WL. The quality of American life. New York: Russell Sage Foundation, 1976.
11. Barrerra M, Sandler IN, Ramsay TB. Preliminary development of a social support scale. Am J Community Psychol 1981;9:435.
12. Folkman S, Lazarus RS. If it changes it must be process: study of emotion and coping during three stages of a college examination. J Pers Soc Psychol 1985;48:150.
13. Spielberger CD. Manual for the State-Trait Anxiety Scale. Palo Alto: Consulting Psychologists Press, 1983.
14. Beck AT, Beamesdeerer A. Assessment of depression: the depression inventory. Pharmacopsychiatria 1976;7:51.
15. Mahlstredt PP. The psychological component of infertility. Fertil Steril 1985;43:335.
16. Lips HM. Somatic and emotional aspects of the normal pregnancy experience: the first 5 months. Am J Obstet Gynecol 1982;142:524.
17. Dennerstein L, Morse C. Psychological issues in IVF. Clin Obstet Gynaecol 1985;12:835.

Detecting changes in functional ability in women with premenstrual syndrome

Barbara W. Posthuma, O.T.(C), M.Ed., Martin J. Bass, M.D., Shelley B. Bull, Ph.D., and Jeffrey A. Nisker, M.D.

London, Ontario, Canada

There has been a lack of objective assessment of the disruptive effect that the symptomatology of premenstrual syndrome has on the functional performance of affected women. This study assessed the functional performance of women with premenstrual syndrome at four phases of the menstrual cycle. Twelve women with premenstrual syndrome and nine asymptomatic women were tested on four functional instruments at the menstrual, early follicular, early luteal, and late luteal phases. Two tests of a paper-and-pencil type measured perceptual parameters, and two tests involving manipulation of objects measured manual dexterity. Mean performance on the Crawford Small Parts Dexterity Test—Part II, which tests fine motor function, was better at the late luteal testing, compared to the early follicular testing, in asymptomatic women but was worse in the women with premenstrual syndrome. This difference was statistically significant ($p = 0.015$). No significant differences between groups in performance changes were observed for the other functional tests. The Crawford Small Parts Dexterity Test–Part II is an objective measure that is potentially valuable in therapeutic trials involving patients with premenstrual syndrome. (Am J Obstet Gynecol 1987;156:275-8.)

Key words: Premenstrual syndrome, functional abilities, manual dexterity

Women with premenstrual syndrome experience both psychological and somatic symptomatology.[1] The question has been raised whether such symptoms may impact on the ability of women to function in the work place.[2] Certain functional abilities have been assessed throughout the menstrual cycle in normal women.[3-5] Although significant cyclic changes were not found in reaction times, Kopell et al.[5] observed that time estimation varied significantly and Stocker[6] found variability in fine and gross motor performance in normal women over the menstrual cycle. The present study was conducted to identify a test that objectively measured changes in functional ability and then to compare the functional ability of women with premenstrual syndrome to that of unaffected women.

Subjects and methods

Subjects who were recruited through newspaper and bulletin board advertisements as well as physician referral were required to meet the following initial criteria: (1) were 28 to 40 years of age; (2) had at least one viable pregnancy; (3) were not taking medications; (4) were free of any manual handicap; (5) had regular menstrual cycles of 26- to 30-day intervals. Then, with use of a symptom questionnaire, subjects rated their emotional symptoms (weepy, depressed, lose temper, irritable, bitchy) and their somatic symptoms (headache, fatigue, bloating, breast swelling, cramping, pain) from absent to marked on a four-point scale throughout the menstrual cycle. Women who experienced moderate or severe symptoms (ratings of 2 or 3) in the luteal phase; which markedly diminished with the menses (ratings of 0 or 1), were considered to be potential premenstrual syndrome subjects. Women with mild or absent symptoms throughout the cycle (ratings of 0 or 1) were accepted as potential control subjects.

To further verify the diagnoses of premenstrual syndrome, all subjects kept a daily diary, recording symptoms and basal body temperatures over two complete menstrual cycles. All diaries were then reviewed in a blinded fashion by one of us (J. A. N.), who categorized the subjects as definitely having premenstrual syndrome, definitely not having premenstrual syndrome, or uncertain. During these 2 months 12 of 28 potential premenstrual syndrome subjects consistently rated their symptoms throughout predictable ovulatory menstrual cycles as marked or severe in the late luteal phase and as absent or mild immediately following the onset of menses. These 12 subjects were then accepted with reasonable assuredness as having premenstrual syn-

From the Departments of Occupational Therapy, Family Medicine, Epidemiology and Biostatistics, and Obstetrics and Gynaecology, The University of Western Ontario.

This research was supported by Ontario Ministry of Health Research Grant No. 01185.

Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.

Reprint requests: Professor Barbara W. Posthuma, Department of Occupational Therapy, Health Sciences Centre, The University of Western Ontario, London, Ontario, Canada N6A 5C1.

Table I. Changes in performance between early follicular and late luteal testings of women with premenstrual syndrome compared to control group

Instrument	*F test (df = 1, 19)*
MacQuarrie Test for Mechanical Ability	1.66
Revised Minnesota Paper Form Board	0.11
Minnesota Rate of Manipulation	0.18
Crawford Small Parts Dexterity Test	5.14*
Crawford Small Parts Dexterity Test—Part II	7.17†

*$p < 0.05$.
†$p < 0.01$.

drome. The accepted premenstrual syndrome subjects all demonstrated both somatic and psychological symptoms, at least one of which was rated as severe. Of 15 potential control subjects, nine who had consistently recorded absent or mild symptoms throughout predictable ovulatory menstrual cycles were considered to be free of premenstrual syndrome and were accepted as controls.

Four functional instruments were chosen for assessment. The MacQuarrie Test for Mechanical Ability[7] and the Revised Minnesota Paper Form Board[8] are paper-and-pencil tests that measure perceptual parameters. The Crawford Small Parts Dexterity Test[9] and the Minnesota Rate of Manipulation Test[10] require subjects to handle objects singly or in combination and measure manual dexterity. All these tests have been standardized, and norms are available for many groups.

Subjects completed all four tests at four specific phases over the menstrual cycle: (1) initial menstruation, days 3 to 5; (2) early follicular, days 6 to 9; (3) early luteal, days 17 to 21; (4) late luteal, days 25-28; they then had a second menstrual assessment. Two different orders of the four test presentations were established and assigned in turn within each group. The tester was blind to the status of each subject.

A general framework for analysis was provided by the repeated-measures analysis of variance model.[11, 12] This model permitted comparison of the two groups with respect to changes between menstrual phases while taking into account the fact that measurements were repeated on each subject on several occasions. The relative magnitude of individual variance as expressed in the intraclass correlation coefficient provided an estimate of reliability.[12] The model assumptions of sphericity were also confirmed.[13] There was no evidence of order effects.

Results

Of the four instruments investigated, only the Crawford Small Parts Dexterity Test showed a significant difference between women with premenstrual syndrome and control subjects ($p < 0.05$). A decrease in functional performance was observed for premenstrual syndrome subjects in the late luteal phase response relative to that at the early follicular phase testing, when compared with control subjects (Table I). Part II of the Crawford Small Parts Test (with a reliability coefficient of 0.66) was primarily responsible for the significant interaction ($p = 0.015$) between clinical group and menstrual phase (Fig. 1). Initially this test took a mean of 10 minutes, 45 seconds to complete with a range of 8 minutes, 27 seconds to 12 minutes, 45 seconds. The standard error bars in Fig. 1 are given to indicate the level of variability in the scores but are not appropriate to make comparisons between groups because of the repeated-measures design.

Although there were significant learning effects for all the tests, there was no evidence of differences in learning effects between the two groups. The group by phase interaction was nonsignificant in an analysis of variance model that included the scores at the two menstrual phases and the early follicular phase.

The two groups were similar in the mandated age and pregnancy categories but were nonsignificantly different in smoking status and occupation, which could account for the differences in group scores at baseline. The effects of full-time employment compared to part-time employment or homemaker status was controlled for in order to assess the influence of life circumstances on the results. On stratification for working status, the difference between cases and controls persisted in the score changes between the two phases.

The distribution of individual subject dexterity changes for the Crawford Small Parts Dexterity Test—Part II between the early follicular and late luteal phases is illustrated in Fig. 2. A positive value indicates an improvement in score whereas a negative value indicates a worsening in score. Because of learning effects, the score changes tend to be positive. With use of zero as a cut-off value, a sensitivity of 58% and a specificity of 78% were obtained. It should be noted that of the nine subjects who scored lower at the luteal phase, all seven premenstrual syndrome subjects had decreases of >30 units while the two subjects without premenstrual syndrome had decreases of <15 units.

Post hoc power analysis on the other three instruments indicated that the sample size was large enough to detect group differences in mean score changes of one standard error with power of at least 80%. Estimates of reliability for the four instruments ranged from 0.65 to 0.80, which are comparable to those reported in the literature.

Comment

Classic premenstrual syndrome presents as a combination of somatic and psychological symptoms during

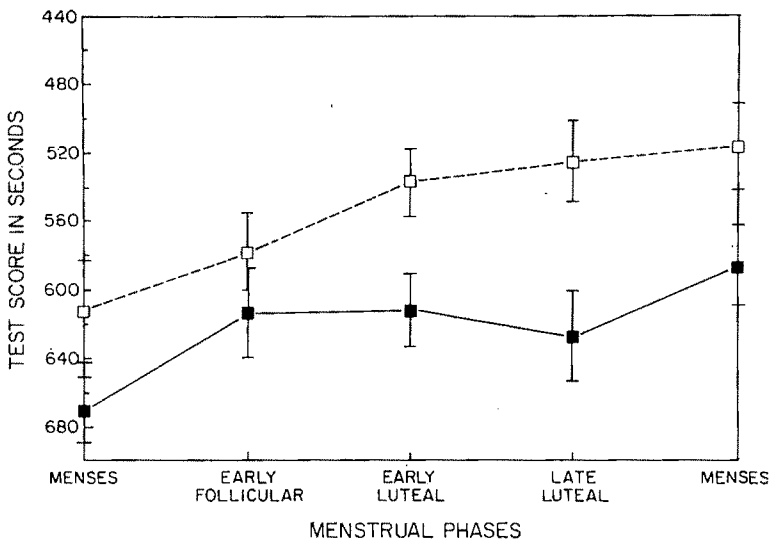


Fig. 1. Group mean profiles of subjects with premenstrual syndrome (■) and controls (□) for the Crawford Small Parts Dexterity Test–Part II. *Bars* indicate standard error of the group means.

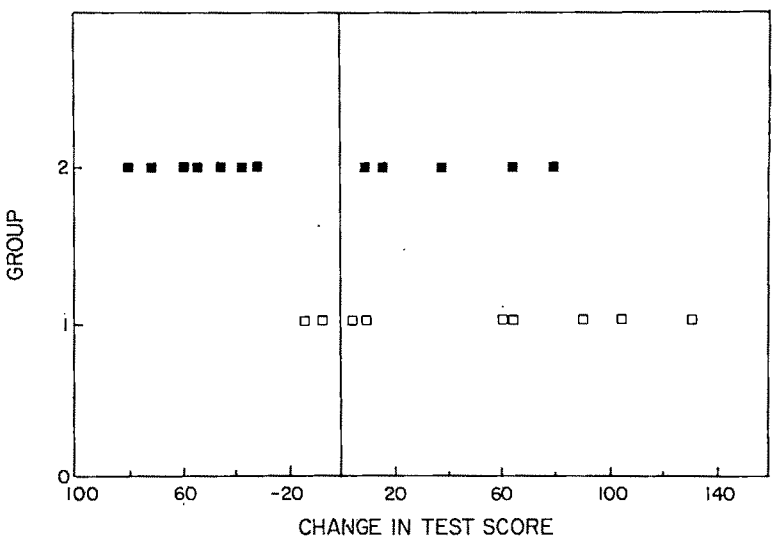


Fig. 2. Distribution of individual score changes in Crawford Small Parts Dexterity Test—Part II between the early follicular and late luteal phases of subjects with premenstrual syndrome (■) and controls (□).

the late luteal phase of the menstrual cycle, with an amelioration of symptoms during menses. In the present study women with premenstrual syndrome did not demonstrate the learning effect in performance on the Crawford Small Parts Dexterity Test–Part II found in the control subjects between the early follicular and the late luteal phase of the cycle. In fact, their performance decreased between these two phases. This test requires manual dexterity in the form of bilateral coordination, balance, concentration, control, and perseverance as 36 small screws are turned into a board with a narrow slotted screwdriver.

The other functional tests did not show differences between the groups in mean score changes. The movements involved in the Minnesota Rate of Manipulation Test require more gross motor performance than fine motor performance. Similarly, the three subtests of the MacQuarrie test assessing mechanical ability do not require bilateral coordination or fine balance and control as does the Crawford Small Parts Dexterity Test—Part II. In addition, since the MacQuarrie tests are shorter in duration, they are less likely to produce fatigue or frustration or to require the same degree of perseverance. The other subtests of the MacQuarrie test along with the Revised Minnesota Paper Form Board primarily test spatial relationships that, based on the present results, do not appear to be disrupted by the menstrual cycle fluctuation.

Zimmerman and Parlee,[14] with use of normal subjects, tested arm-hand steadiness, reaction time, galvanic skin response, time estimation, and digit-symbol substitution, observing that only the arm-hand steadiness test showed statistically significant fluctuations over the phases of a menstrual cycle. These normal subjects showed decreased steadiness during the premenstrual phase when compared with the early luteal phase.

Functional ability over the menstrual cycle has been tested in normal women and generally only in a stimulus-response situation.[4, 5, 15, 16] For the most part these studies do not demonstrate a relationship between performance and menstrual phase.[4, 5, 15, 16] It may be argued that one might expect subjects to display higher functional abilities in a stimulus-response test than one would find in a self-motivating response situation. For example, in the Crawford Small Parts Dexterity Test—Part II, the subject must rotate small screws into a board; since there are no signals for each movement, the subject must motivate herself to perform the task. In a stimulus-response test the subject is prodded to perform by the stimulus so there would be increased performance because of reflex action. A self-motivating type of test, such as the Crawford Small Parts Dexterity Test, may represent a more realistic facsimile of daily functional abilities.

This study has identified a test that is sensitive to decreased bilateral fine motor function in women with premenstrual syndrome during the late luteal phase of the cycle, while showing no such phase effect in normal women. Only in this one bilateral fine motor area did women with premenstrual syndrome demonstrate less functional ability than normal women. Because of the limited number of subjects in this pilot study it was not possible to explore fully whether there are subgroups of the subjects with premenstrual syndrome who are differentially affected. The Crawford Small Parts Dexterity Test—Part II is an objective measure that is potentially valuable in therapeutic trials involving patients with premenstrual syndrome.

REFERENCES

1. Abplanalp JM. Premenstrual syndrome: a selective review. Women's Health 1983;8:107.
2. Sommer B. Menstrual cycle changes and intellectual performance. Psychosom Med 1972;34:263.
3. Hutt SJ, Frank G, Mychalkiev W, Hughes M. Perceptual motor performance during the menstrual cycle. Horm Behav 1980;14:116.
4. Pierson WR, Lockhart A. Effect of menstruation on simple reaction and movement time. Br Med J 1963;1:796.
5. Kopell BS, Lunde DT, Clayton BB, Moos RH. Variations in some measures of arousal during the menstrual cycle. J Nerv Ment Dis 1969;148:180.
6. Stocker JM. Motor performance and state anxiety at selected stages of the menstrual cycle. Dis Abst Int 1974; 34:3971.
7. MacQuarrie TW. MacQuarrie Test for Mechanical Ability. Monterey, California: McGraw-Hill, 1953.
8. Likert R, Quasha WH. Revised Minnesota Paper Form Board, series BB. New York: Psychological Corporation, 1969.
9. Crawford JE, Crawford DM. Crawford Small Parts Dexterity Test. New York: Psychological Corporation, 1981.
10. American Guidance Service. The Minnesota Rate of Manipulation Test: examiners manual. Circle Pines, Minnesota: AGS Inc., 1957.
11. Winer BJ. Statistical principles in experimental design. 2d ed. New York: McGraw-Hill, 1971.
12. Bock RD. Multivariate statistical methods in behavioral research. New York: McGraw-Hill, 1975.
13. Dixon WJ, Brown MB., eds. Biomedical computer programs—BMOP 2V: analysis of variance and covariance with repeated measures. Berkeley, California: University of California Press, 1982.
14. Zimmerman E, Parlee MB. Behavioral changes associated with the menstrual cycle: an experimental investigation. J Appl Soc Psychol 1973;3:335.
15. Southam AL, Gonzage FP. Systemic changes during the menstrual cycle. Am J Obstet Gynecol 1965;91:142.
16. Loucks J, Thompson H. Effects of menstruation on reaction time. Res Quart 1968;39:407.

Endocrine changes after laparoscopic ovarian cautery in polycystic ovarian syndrome

Ellen Greenblatt, M.D., and Robert F. Casper, M.D.
London, Ontario, Canada

Recently, laparoscopic ovarian cautery has been described as a method of ovulation induction in women with polycystic ovarian disease. In an attempt to determine the mechanism of action, serum levels of androstenedione, testosterone, luteinizing hormone, follicle-stimulating hormone, and estradiol were determined before and after the laparoscopic ovarian cautery in six women with polycystic ovarian disease who had failed to ovulate with clomiphene citrate and human chorionic gonadotropin. Six regularly cycling women undergoing laparoscopy for investigation of infertility or tubal ligation served as controls. In patients with polycystic ovarian disease but not in controls, serum androstenedione, testosterone, estradiol, and luteinizing hormone significantly decreased to nadir levels on postoperative days 3 and 4. In contrast follicle-stimulating hormone levels rose after operation. These results resemble those reported after ovarian wedge resection. Of the six treated women, five ovulated postoperatively and four conceived. Laparoscopic ovarian cautery appears to be a promising alternative treatment for patients with polycystic ovarian disease in whom initial medical management fails. (Am J Obstet Gynecol 1987;156:279-85.)

Key words: Polycystic ovary, laparoscopy, ovarian cautery

Patients with chronic anovulation because of polycystic ovarian disease generally respond to clomiphene citrate administration for induction of ovulation. However, even with the use of increasing doses of clomiphene with or without the addition of human chorionic gonadotropin, 10% to 15% of women remain anovulatory.[1] For these patients, ovulation induction can be difficult, requiring parenteral treatment with human menopausal gonadotropin–human chorionic gonadotropin or pure follicle-stimulating hormone, both of which are costly and require careful hormonal and ultrasound monitoring to prevent a significant incidence of ovarian hyperstimulation.

Alternatively, surgical treatment by ovarian wedge resection, although successful in inducing ovulatory cycles,[2] has largely been abandoned because of the need for laparotomy and the potential for development of pelvic adhesions.[3] Although not thoroughly investigated, recent work suggests that application of microsurgical techniques for ovarian wedge resection may again render this treatment acceptable.[4] The mechanism by which ovarian wedge resection can induce ovulation is unclear; however, investigations of hormonal changes after operation have demonstrated dramatic decreases in serum androgen levels together with equivocal changes in estradiol and luteinizing hormone levels.[5, 6] Recently a less extensive surgical technique involving laparoscopic ovarian cautery has been described for the treatment of anovulation in patients with polycystic ovarian disease.[7] Clinical results appear promising with a high rate of successful ovulation and conception occurring without the need for intensive patient monitoring.

The present study was proposed to evaluate the clinical and hormonal response to laparoscopic ovarian cautery in a selected group of women with polycystic ovarian disease who consistently failed to ovulate with clomiphene citrate and human chorionic gonadotropin. In particular, we examined serum levels of testosterone, androstenedione, estradiol, follicle-stimulating hormone, and luteinizing hormone before and after ovarian cautery to determine if changes similar to those after ovarian wedge resection occurred.

Material and methods

Six women between the ages of 23 and 36 with clinical and biochemical findings consistent with polycystic ovarian syndrome were studied. Criteria for inclusion were the following: infertility secondary to anovulation as indicated by amenorrhea, elevated serum luteinizing hormone levels and normal to low serum follicle-stimulating hormone levels relative to follicular phase values in normal women, clinical evidence of androgen excess (acne, hirsutism), and slightly elevated androgen levels (androstenedione and testosterone). All patients

From the Medical Research Council Group in Reproductive Biology and the Department of Obstetrics and Gynaecology and Physiology, The University of Western Ontario.
Supported by a grant from the Medical Research Council of Canada.
Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.
Reprint requests: Robert F. Casper, M.D., University Hospital, 339 Windermere Road, London, Ontario, Canada N6A 5A5.

February 1987
Am J Obstet Gynecol



Fig. 1. Polycystic ovary before *(left)* and after *(right)* laparoscopic ovarian cautery.

Table I. Patient profiles

Age (yr)	*Type of infertility*	*Duration of infertility (yr)*	*Height (cm)*	*Weight (kg)*	*Hirsutism*	*Preoperative LH/FSH ratio*	*Preoperative T (ng/ml)*	*Preoperative A (ng/ml)*
31	Primary	4 years	154.6	78.6	Moderate	2.45	2.02	3.49
36	Secondary	2½ years	154.2	77.9	Mild	2.38	0.98	4.24
25	Primary	6 years	163.2	59.8	Moderate	6.69	1.26	5.79
23	Primary	2 years	164.6	61.9	Moderate	2.42	1.40	4.32
25	Primary	2 years	160.7	62.2	Moderate	3.69	0.72	3.40
24	Primary	2 years	161.2	68.0	Moderate	5.53	0.75	6.96

LH = Luteinizing hormone; FSH = follicle-stimulating hormone; T = testosterone; A = androstenedione.

had failed to ovulate after increasing doses of clomiphene citrate up to a maximum of 150 mg per day with the addition of human chorionic gonadotropin (5000 IU) on day 16 for at least three cycles. The clinical characteristics of these women are shown in Table I. All were being considered for treatment with human menopausal gonadotropin. This study was approved by the University of Western Ontario health sciences committee on human research and informed consent was obtained.

Laparoscopic ovarian cautery was performed with the use of a three-puncture technique. The laparoscope was introduced through a subumbilical incision and a grasping forceps was introduced suprapubically to stabilize the ovary by grasping the ovarian ligament. After assessment of the pelvic structures and tubal patency, laparoscopic scissors connected to a unipolar electrocautery unit were inserted through a third puncture site in the lower abdomen. Eight to ten cautery points 3 to 4 mm in diameter were created in each ovary (Fig. 1) with a current of 4 mA applied through the laparoscopic scissors. Cautery was continued until penetration of the ovarian capsule and cortex was achieved.

The women were studied before and after operation to assess serum levels of the following hormones: luteinizing hormone, follicle-stimulating hormone, estradiol, androstenedione, testosterone, and progesterone. Blood samples were drawn before operation, daily after operation for 2 weeks, and then every 2 days for a total of 4 weeks or until the first menses. All patients were evaluated clinically for up to 18 months.

Six regularly cycling control subjects aged 21 to 33 were selected from women undergoing either diagnostic laparoscopy or laparoscopic tubal ligation for study of the effects of anesthesia and laparoscopy on the serum levels of the above hormones. Two patients were in the early follicular phase and four were in the luteal phase. One preoperative and seven daily postoperative blood samples were obtained.

Blood samples were allowed to clot at room temperature and the serum was separated and frozen at −20° C until assayed. All samples from each patient were run in the same assay. Serum levels of 17β-estradiol and progesterone were determined by previously described radioimmunoassay techniques.[8,9] Luteinizing hormone and follicle-stimulating hormone (Amer-

sham, Oakville, Ontario, Canada; double-antibody iodine 175 radioimmunoassay) and androstenedione and testosterone (Diagnostic Products Corp, Los Angeles, California; solid-phase iodine 125 radioimmunoassay) were measured with the use of commercially prepared kits. Student's *t* test and analysis of variance were used to analyze the data.

Results

There were no complications from the laparoscopic ovarian cautery and all patients were discharged home within 3 hours. Five of the six patients ovulated and menses occurred between 24 and 33 days after operation. Four continued to have regular menses and all of these women became pregnant. One patient ovulated only once after operation and then became amenorrheic but responded to clomiphene citrate, 50 mg, and is ovulating regularly on this dosage. At the time of laparoscopy, this patient had unilateral tubal occlusion and minimal pelvic adhesions on the same side. One patient did not respond and was treated with human menopausal gonadotropin.

Serum concentrations of androstenedione and testosterone before operation and in the first 10 days after operation in the patients with polycystic ovarian disease are shown in Fig. 2. Before operation, the mean (±SEM) serum androstenedione level was 4.77 ± 0.69 ng/ml and that of testosterone was 1.16 ± 0.15 ng/ml. A rapid and significant ($p < 0.01$) decrease in the levels of both hormones occurred after operation, with nadir values of 1.95 ± 0.4 and 0.57 ± 0.1 ng/ml reached on days 4 and 3, respectively. Serum concentrations of luteinizing hormone, follicle-stimulating hormone, and estradiol are shown in Fig. 3. After ovarian cautery, a fall in the serum luteinizing hormone level from 37.6 ± 3.2 mIU/ml before operation to 27.7 ± 2.5 mIU/ml ($p < 0.05$) on day 3 was seen and was followed by a preovulatory surge in five of the six patients. In contrast, the follicle-stimulating hormone levels were seen to rise from a preoperative value of 5.27 ± 0.37 mIU/ml to a peak on postoperative day 2 ($p < 0.01$) of 7.43 ± 0.83 mIU/ml before a return to preoperative values by day 10. Preoperative estradiol levels in the patients with polycystic ovarian disease were 90.2 ± 5 pg/ml; values fell to a nadir of 50.7 ± 6.1 pg/ml ($p < 0.01$) on the fourth day after ovarian cautery. In contrast, control subjects had significantly ($p < 0.01$) lower preoperative androstenedione and testosterone levels (1.6 ± 0.20 and 0.48 ± 0.08 ng/ml, respectively), and these did not change after operation (Fig. 4).

Individual hormone responses in a single patient during her first ovulatory cycle demonstrates clearly the temporal sequence of events after laparoscopic ovarian cautery (Fig. 5). The rapid fall in androgen levels after operation is dramatic and precedes changes in the other hormones. In the single patient who did not respond to ovarian cautery, declines in the concentrations of serum androstenedione, testosterone, and estradiol were similar to those in the women who ovulated after the procedure, but no fall in the serum luteinizing hormone level was observed after operation and the postoperative rise in follicle-stimulating hormone was slower and less marked. No preovulatory peak of serum estradiol or luteinizing hormone occurred.

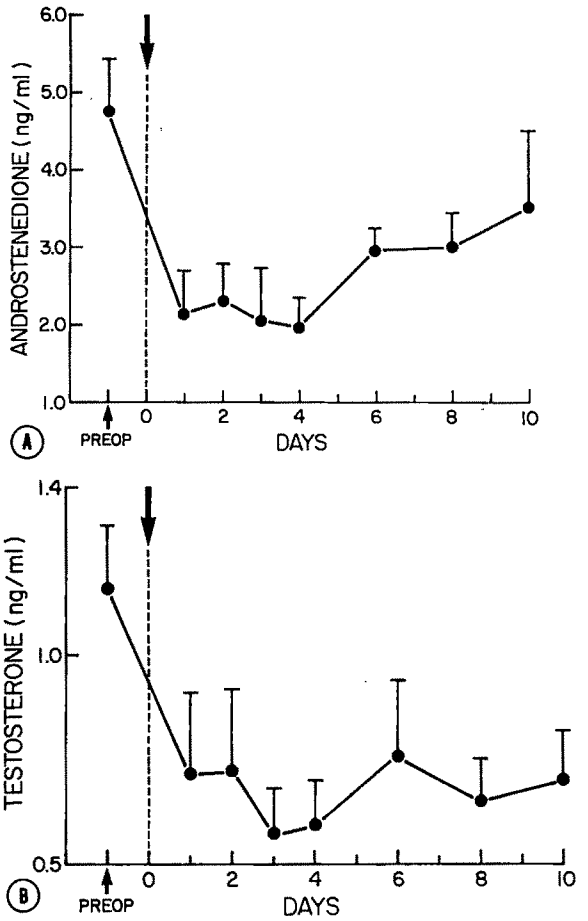


Fig. 2. Preoperative and postoperative serum androgen concentrations in the six patients with polycystic ovarian disease undergoing ovarian cautery. *Arrow* indicates day of operation. *A*, Mean ± SEM serum androstenedione concentrations before operation and 10 days after operation. *B*, Mean ± SEM serum testosterone concentrations before operation and 10 days after operation.

Comment

Treatment of infertility in polycystic ovarian disease is aimed at reversing chronic anovulation. This group of patients is one of the most sensitive to ovulation

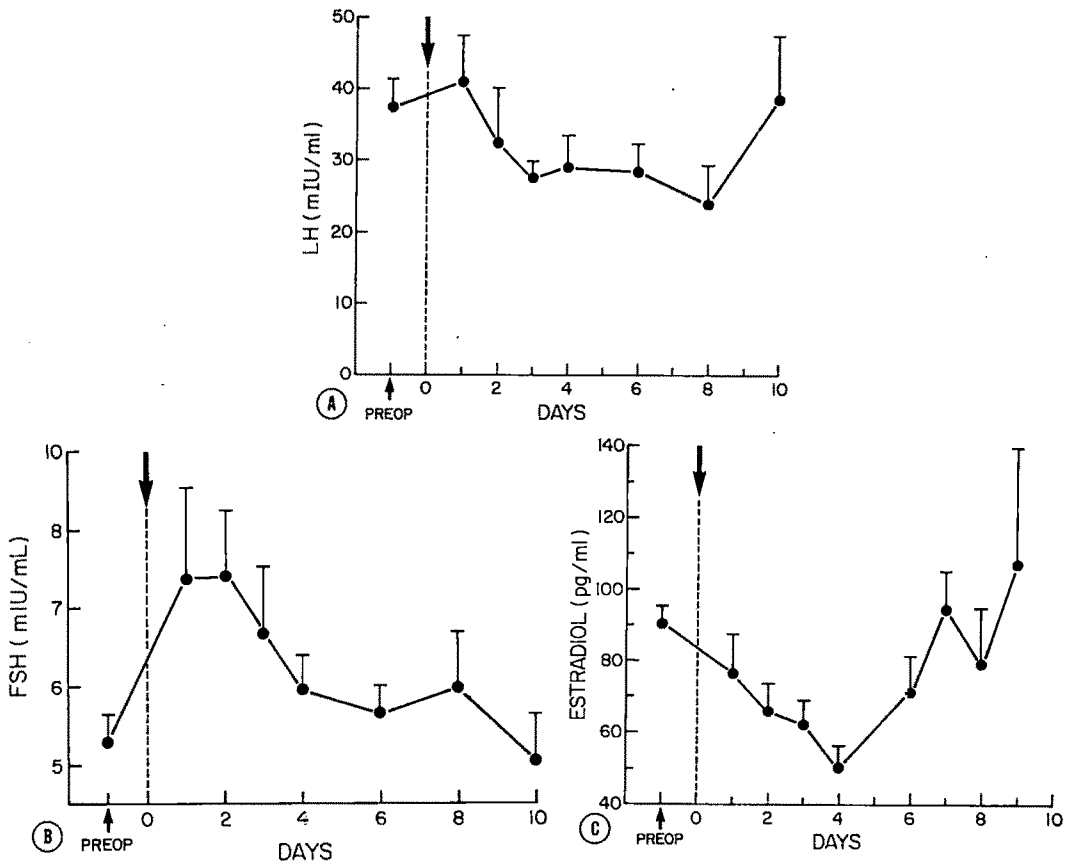


Fig. 3. Preoperative and postoperative gonadotropin and estradiol concentrations in the six patients with polycystic ovarian disease undergoing ovarian cautery. *Arrow* indicates day of operation. *A*, Mean ± SEM serum luteinizing hormone concentrations before operation and 10 days after operation. *B*, Mean ± SEM serum follicle-stimulating hormone concentrations before operation and 10 days after operation. *C*, Mean ± SEM serum estradiol concentrations before operation and 10 days after operation.

induction with clomiphene citrate with response rates of 80% to 90%. For clomiphene citrate failures, the next line of treatment is generally human menopausal gonadotropin and human chorionic gonadotropin with successful induction of ovulation in 75% to 95% of cases.[1] However, human menopausal gonadotropin–human chorionic gonadotropin therapy is costly and demands extensive monitoring to prevent the high incidence of hyperstimulation and multiple births in this group of women.

An alternative means of ovulation induction involves ovarian wedge resection, which was popular at the time that the syndrome was described in 1935.[10] Reports of the incidence of successful ovulation after ovarian wedge resection varies quite widely in the literature with a range of 6% to 95% and a mean of 80%.[2] However, ovarian wedge resection may not be a permanent cure for polycystic ovarian disease,[3] and this treatment has fallen into disfavor because of the significant incidence of postoperative peritubal and periovarian adhesions, which has not been adequately reevaluated in light of more recent microsurgical techniques. The mechanism by which ovarian wedge resection induces ovulation is not known. Early investigators believed that a mechanical barrier to ovulation was removed by incision of the thickened ovarian capsule.[10] However, it is probable that this explanation is too simplistic and several investigators have studied the hormonal alterations associated with ovarian wedge resection in an attempt to determine the mechanism of action.[5,6] The most consistent finding in these studies is a dramatic fall in serum androgen levels in the first few days after operation. Judd et al.[5] studied eight patients with polycystic ovarian disease undergoing wedge resection and described a dramatic decrease in serum androstenedione and testosterone concentrations occurring in the first 3 days after operation. A less dramatic but significant decrease in estradiol with a nadir on postoperative day 3 was also noted. No consistent postoperative alterations in gonadotropins were observed. These investigators suggested that the decreased androgen levels may have removed an intraovarian block

to follicular maturation or that the hyperemia associated with postoperative inflammation allowed increased delivery of gonadotropins. Katz et al.,[6] in a study of two patients, described a similar decrease in testosterone after operation but also observed a decrease in luteinizing hormone levels such that the elevated preoperative luteinizing hormone: follicle-stimulating hormone ratio was normalized. Katz et al. postulated that the postoperative decrease in estradiol and androgens eliminated a persistent positive feedback effect of these steroids on the pituitary, allowing luteinizing hormone levels to return to normal. The normalization of the follicle-stimulating hormone: luteinizing hormone ratio may then allow follicular maturation to proceed.

The present study was designed to characterize the hormonal effects of laparoscopic ovarian cautery as first described by Gjonnaess.[7] In his report on 62 women with polycystic ovarian disease, evidence of ovulation was demonstrated after ovarian cautery in 92% of patients. Of 35 women desiring pregnancy, 69% became pregnant after cautery alone with a total of 80% conceiving after cautery or cautery with the addition of clomiphene. In a subsequent study, Asbjorn and Gjonnaess[11] measured hormonal alterations after laparoscopic ovarian cautery. These authors measured the same hormones 1 day after operation, weekly for 4 weeks, and then periodically up to 1 year and documented ovulation in 72% of 59 women within 4 weeks of cautery. In contrast to the results of the present study, an immediate rise in luteinizing hormone 1 day after operation was seen and this was followed by a gradual fall to levels significantly below preoperative values by day 4. The follicle-stimulating hormone concentrations in these ptients increased significantly on day 1 after operation. Serum concentrations of androstenedione, testosterone, and dihydrotestosterone fell significantly on postoperative day 1 and by 4 weeks after operation they were still lower than pretreatment values.

In the present study, we have demonstrated a dramatic fall in androgen levels in the early postoperative period that is in keeping with similar changes described after wedge resection. However, in contrast to the procedure of wedge resection in which up to 50% of each ovary is removed, only a small percentage of the ovarian cortex and subcortical tissue is destroyed by ovarian cautery. An even smaller removal of ovarian tissue such as occurs with laparoscopic ovarian biopsy can also occasionally induce ovulation in patients with polycystic ovarian disease.[12]

Erickson et al.[13] have demonstrated that dispersed granulosa cells from women with polycystic ovarian disease, when supplemented with follicle-stimulating hormone, have the ability to aromatize androstenedione to estradiol, similar to that of cells from equal-sized follicles from normal ovaries. There does not therefore seem to be an inherent aromatase defect in the polycystic ovary but rather a relative lack of follicle-stimulating hormone. The hypothalamus and pituitary in patients with polycystic ovarian disease can also respond appropriately to steroid feedback signals.[14] McNatty et al.[15] have demonstrated the marked androgen-producing capacity of stromal tissue in polycystic ovaries, and follicular fluid from polycystic ovaries has been found to contain high concentrations of androgens.[13, 15] Therefore it is possible that local factors such as elevated intraovarian androgen levels may contribute to the inhibition of follicular development[16] as seen in patients with polycystic ovarian disease.

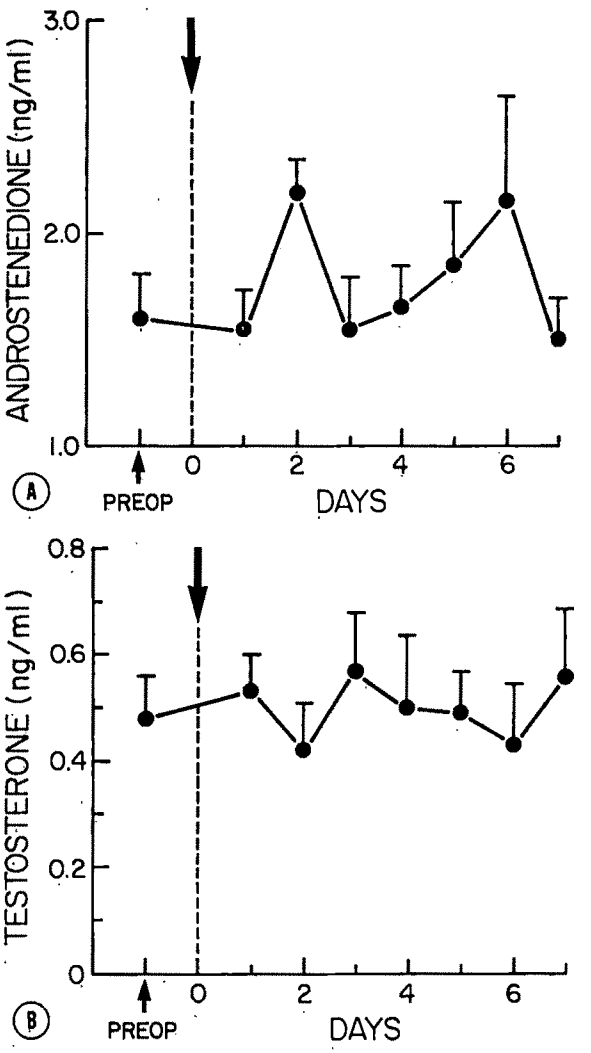


Fig. 4. Preoperative and postoperative serum androgen concentrations in the six control patients undergoing laparoscopy. *Arrow* indicates day of operation. *A*, Mean ± SEM serum androstenedione concentrations before operation and for 1 week after operation. *B*, Mean ± SEM serum testosterone concentrations before operation and for 1 week after operation.

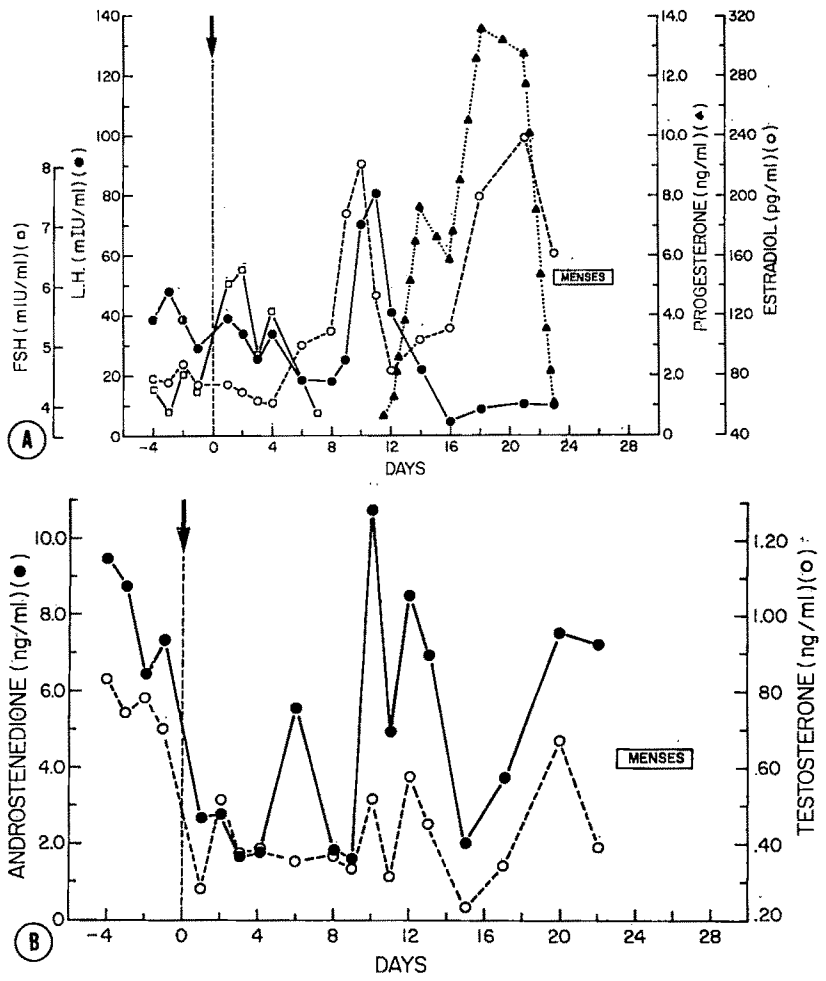


Fig. 5. Example of the pattern of hormonal changes seen in one patient with polycystic ovarian disease treated with laparoscopic ovarian cautery up to the time of the first menses. *A*, Serum concentrations of luteinizing hormone (•), follicle-stimulating hormone (□), estradiol (○), and progesterone (▲) before and after operation. Note preovulatory rise in serum estradiol level and luteinizing hormone surge. *B*, Serum concentration of androstenedione (•), and testosterone (○) before and after operation. Note marked fall in both androgen levels that preceded changes in levels of hormones in *A*.

Trauma to the ovary in various forms (wedge resection, ovarian biopsy, and cautery) may impair local production of androgens. A decrease in androgen levels may result in lowered peripheral conversion of androgen to estrogen and decreased positive feedback on luteinizing hormone production centrally. Negative feedback on follicle-stimulating hormone secretion may also be diminished, allowing follicle-stimulating hormone levels to rise. This may be secondary to a decrease in peripheral estrogens and possibly due to a decrease of inhibin from the cauterized ovary. Lower luteinizing hormone levels might then decrease stromal androgen production and along with increasing follicle-stimulating hormone levels allow follicular development to proceed on to ovulation.

Our data support this hypothesis by demonstrating a drop in peripheral androgen levels preceding the fall in serum estradiol and luteinizing hormone levels. At the same time, follicle-stimulating hormone levels rise, in agreement with Katz et al.,[6] who noted a rise in follicle-stimulating hormone levels after ovarian wedge resection. One may speculate that the fall in local androgen levels and the decrease in the luteinizing hormone: follicle-stimulating hormone ratio would then remove the block to follicular maturation and allow progression to ovulation.

In conclusion, our data demonstrate that laparoscopic ovarian cautery is characterized by dramatic postoperative decreases in androstenedione and testosterone as well as smaller but significant decreases in estradiol and luteinizing hormone. Follicle-stimulating hormone values rise after operation, and this contributes to a decrease in the abnormally elevated luteinizing hormone: follicle-stimulating hormone ratio. These

changes are not a result of anesthesia and laparoscopy alone.

Preliminary clinical results are encouraging. Furthermore, the procedure is relatively simple, adds minimal morbidity to diagnostic laparoscopy, and avoids the complication of hyperstimulation associated with human menopausal gonadotropin–human chorionic gonadotropin therapy. Long-term effects are as yet unknown and the duration of the clinical effects as well as biochemical effects remains to be determined. Larger numbers will have to be treated to ensure the safety of this procedure. Laparoscopic ovarian cautery holds promise for infertile women with polycystic ovarian disease who experience failure of initial medical management.

We wish to thank Wendy Powell and Anne-Marie Powell for excellent technical and nursing assistance.

REFERENCES

1. Franks S, Adams J, Mason H, Polson D. Ovulatory disorders in women with polycystic ovary syndrome. Clin Obstet Gynecol 1985;12:605.
2. Goldzieher JW, Green JA. The polycystic ovary. Clinical and histological features. J Clin Endocrinol Metab 1962; 22:325.
3. Buttram V, Vaquero C. Post-ovarian wedge resection adhesive disease. Fertil Steril 1975;26:874.
4. Eddy CA, Asch RH, Balmaceda JP. Pelvic adhesions following microsurgical and macrosurgical wedge resection of the ovaries. Fertil Steril 1980;33:557.
5. Judd HL, Riggs LA, Anderson DC, Yen SSC. The effects of ovarian wedge resection on circulating gonadotropin and ovarian steroid levels in patients with polycystic ovary syndrome. J Clin Endocrinol Metab 1976;43:347.
6. Katz M, Carr PJ, Cohen BM, Millow RP. Hormonal effects of wedge resection in polycystic ovaries. Obstet Gynecol 1978;51:437.
7. Gjonnaess H. Polycystic ovarian syndrome treated by ovarian electrocautery through the laparoscope. Fertil Steril 1984;41:20.
8. Devane GW, Dzekala NC, Judd HL, Yen SSC. Circulating gonadotropins, estrogens and androgens in polycystic ovarian disease. AM J OBSTET GYNECOL 1975;121:496.
9. Patrick J, Challis J, Natale R, Richardson B. Circadian rhythms in maternal plasma cortisol, estrone, estradiol, and estriol at 34 to 35 weeks' gestation. AM J OBSTET GYNECOL 1979;135:791.
10. Stein IF, Leventhal HI. Amenorrhea associated with bilateral polycystic ovaries. AM J OBSTET GYNECOL 1935; 29:181.
11. Asbjorn A, Gjonnaess H. Hormonal response to electrocautery of the ovary in patients with polycystic ovarian disease. Br J Obstet Gynaecol 1985;92:1258.
12. Yuzpe AA, Rioux JE. The value of laparoscopic ovarian biopsy. J Reprod Med 1975;15:57.
13. Erickson GF, Hsueh AJW, Quigley ME, Rebar RW, Yen SSC. Functional studies of aromatic activity in human granulosa cells from normal and polycystic ovaries. J Clin Endocrinol Metab 1979;49:514.
14. Yen SSC. The polycystic ovary syndrome. Clin Endocrinol 1980;12:177.
15. McNatty KP, Smith DM, Makors A, et al. The intraovarian sites of androgen and estrogen formation in women with normal and hyperandrogenic ovaries as judged by in vitro experiments. J Clin Endocrinol Metab 1980;50:755.
16. Louvet JP, Harman SM, Schreiber JR, Ross GT. Evidence for a role of androgens in follicular maturation. Endocrinology 1975;97:366.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1987 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 156 (January-June) and Vol. 157 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Human chorionic gonadotropin increase in normal early pregnancy

Salim Daya, M.B., Ch.B.*
Hamilton, Ontario, Canada

Serial determinations of human chorionic gonadotropin have been used in evaluating early pregnancies. This has been based on linear regression of logarithm-transformed hCG levels. However, these levels are changing in a curvilinear fashion as pregnancy advances. Consequently the simple linear model does not provide a good fit to the data. To define more precisely the normal values for hCG increase, serial determinations were performed on serum samples obtained from 29 patients who were carrying normally advancing pregnancies. Gestational age was established with basal body temperature records. Three gestational age periods were identified each with a linear increase in hCG. This was translated into hCG doubling times and percentage increase over time. These tables may be used to determine if an early pregnancy is advancing normally. (Am J Obstet Gynecol 1987;156:286-90.)

Key terms: Pregnancy, normal, early chorionic gonadotropin levels

The development of laboratory assays for the detection of pregnancy began almost 60 years ago with the report of a time-consuming bioassay performed in mice.[1] Since then it was discovered that human chorionic gonadotropin (hCG) was the hormone produced in normal pregnancies and consequently a number of biologic, immunologic, and radioreceptor assays were developed to measure this hormone.[2] One significant development was an antiserum specific for the β-subunit of hCG, thereby allowing very low levels of the hormone to be detected without interference from luteinizing hormone.[3] This high level of sensitivity has enabled hCG to be detected by radioimmunoassay as early as 6 days after conception.[4] With increasing availability of this assay the progress of early pregnancy can be monitored with serial determination of hCG.[5-8]

The majority of the reports in the literature have been based on calculating predicted increases in hCG over time using linear regression of logarithmically transformed hCG levels in the first trimester. With use of this method it has been assumed that the rate of rise of hCG is constant in normal early intrauterine pregnancies. However, hCG levels are constantly changing with the rate of rise decreasing as pregnancy advances until a plateau is reached at approximately 9 to 10 weeks' gestation. Simple linear regression of log hCG does not adequately represent this change. Consequently the predictive value for normal pregnancy with use of doubling times based on this method may lack sensitivity. An attempt to improve this has been made with use of smaller sampling intervals of hCG.[8]

Regression analysis is used to establish whether a relationship exists between two variables. For a particular value of the independent variable, the regression line helps to predict a corresponding best estimate of the dependent variable. By plotting a series of hCG levels for each gestational age, a simple linear regression model can provide a best estimate for hCG level at a particular gestational age. Since hCG levels are increasing in pregnancy in a nonlinear way, this model is not suitable in providing estimates of hCG *increase* over time without the knowledge of gestational age. However, this has been the main focus in the literature.

In this study, normal values for the rate of hCG increase in early pregnancy have been defined more precisely and may improve the sensitivity and usefulness of serial determinations of hCG in the early detection of pathologic pregnancies.

Material and methods

Patients undergoing evaluation and treatment at the Infertility Clinic at Chedoke-McMaster Hospital, Hamilton, Canada, were selected for this study after giving informed consent. Serial hCG determinations were performed on serum samples obtained from 29 such patients who had conceived and were carrying normally advancing intrauterine pregnancies as confirmed by ultrasonography. The mean sampling interval was 3.8 days. All these patients have since delivered healthy

From the Department of Obstetrics and Gynecology, McMaster University.
Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.
Reprint requests: Dr. Salim Daya, Department of Obstetrics and Gynecology, McMaster University, 1200 Main St. West, Hamilton, Ontario, Canada L8N 3Z5.
Career Scientist of the Ontario Ministry of Health, Canada.

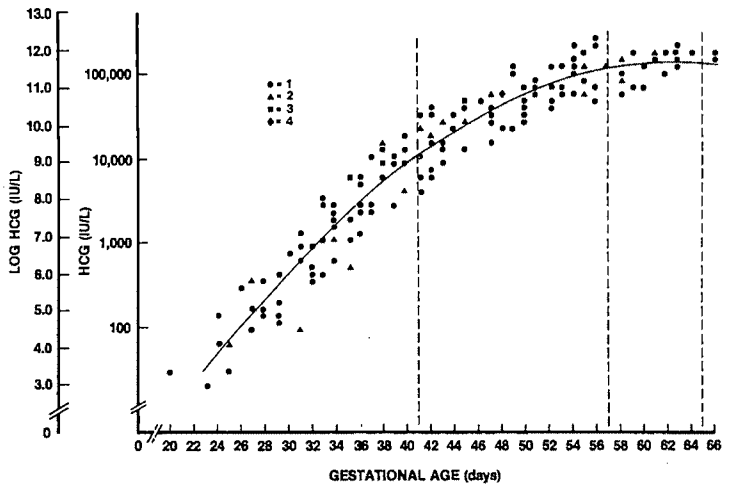


Fig. 1. hCG increase in normal early pregnancy. Quadratic regression line equation is as follows: log hCG = −8.8695 + 0.6530 (gestational age) − 0.0052 (gestational age)^2. *Symbols* correspond to the indicated number of data points.

Table I. Polynomial regression analysis of early pregnancy hCG data*

	F values			
			Improved predictive power	
Regression models	*Overall regression*	*Lack of fit*	*With use of quadratic vs. straight line*	*With use of cubic vs. quadratic*
Straight line	985 ($p < 0.0001$)	2.22 ($p < 0.005$)	176 ($p < 0.001$)	
Quadratic	1109 ($p < 0.0001$)	0.96 ($p > 0.25$)		0.74 ($p > 0.25$)
Cubic	738 ($p < 0.0001$)	0.96 ($p > 0.25$)		

*Total plotted values = 165.

infants at term. Although some patients conceived spontaneously, most required therapeutic intervention in the form of homologous or heterologous insemination, clomiphine citrate, human menopausal gonadotropin, and/or human chorionic gonadotropin. Gestational age was established in all cases by the nadir on basal body temperature recordings.

Radioimmunoassay. Patient serum was separated from clotted blood samples and stored at −20° C. Quantitative measurements of hCG were performed with use of a GammaDab M [iodine 125] β-subunit of hCG radioimmunoassay kit (Travenol-Genentech Diagnostics, Cambridge, Massachusetts). The procedure is a competitive binding assay that uses a precipitating antiserum reagent generated against the β-subunit of the hCG hormone, ^{125}I-labeled hCG, and a highly purified preparation of hCG reagents as control (CA 2426), standard (CA 22002, 22003, 22004, 22006, and 22007) and calibrator/standard (CA 22005). The control reagent is calibrated at 90 mIU/ml, the standards at 5, 10, 25, 100, and 200 mIU/ml, and the calibrator/standard at 50 mIU/ml. The standards and control reagents are calibrated to the World Health Organization's immunoassay standard, the first international reference preparation 75/537 established in 1975, and labeled in international milliunits per milliliter. The values of the controls, standards, and patient serum can be converted directly from international milliunits per milliliter to international units per liter of standard international units. The calculated sensitivity of the assay is 1 to 5 mIU/ml with <5 mIU/ml being taken as evidence for a nonpregnant state. The intrarun variability determined from the mean of 20 simultaneous assays for three samples was 3.2% (range, 2.9% to 3.6%), and the interrun variability determined from the mean of the average of duplicates for 20 separate runs was 7.4% (range, 6.5% to 9.0%).

Statistics. Simple linear regression was performed

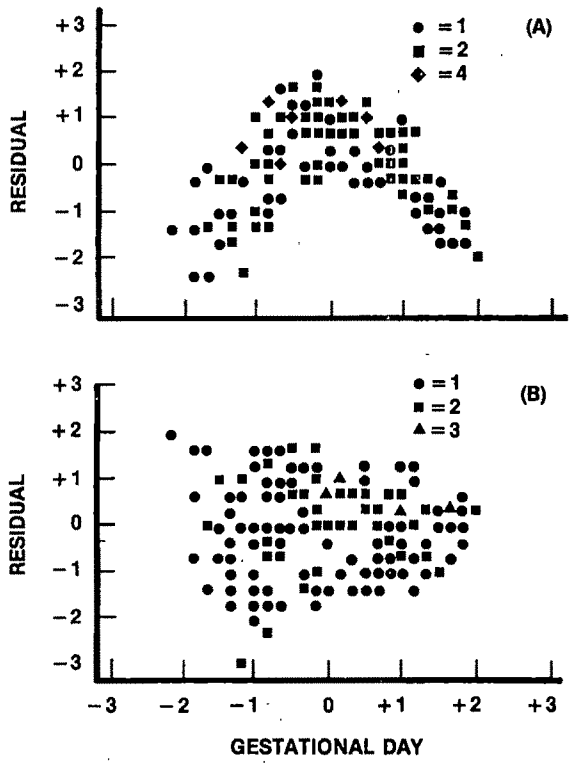


Fig. 2. Standardized scattergrams of residuals. *A*, Simple linear regression model. *B*, Quadratic regression model. *Symbols* correspond to the indicated number of data points.

on the hCG levels for each patient. Three gestational age ranges were specified (given in the Results section) and within each one the mean and lower two standard deviations (SD) were calculated for the slope parameters.

Results

Since some of the patients had received hCG injections to induce ovulation, clearance studies were performed to determine how rapidly 10,000 IU of this hormone could be eliminated from the body. Daily blood samples were taken for hCG determinations from three such patients. It was observed that hCG was no longer detectable 12 days after the initial administration of this hormone. Subsequently in such patients, blood samples for hCG were not drawn earlier than 12 days after the last hCG dose.

Fig. 1 represents a scattergram of log hCG plotted against gestational age. It can be seen that as gestational age advances, the hCG level increases until a plateau is reached at approximately 9 weeks' gestation. The relationship appears to be a curvilinear one despite the logarithmic transformation. To determine which regression model would best fit the data, the following were tested: straight line, quadratic, and cubic. From Table I it is apparent that all models demonstrate a significant overall regression. However, when lack of fit for each model is tested, the simple linear model is not appropriate ($F_{42,121} = 2.22$, $p < 0.005$) whereas both the quadratic and cubic models are appropriate ($F_{41,121} = 0.956$, $p > 0.25$, and $F_{40,121} = 0.961$, $p > 0.25$, respectively). The quadratic model provides significantly more predictive power than that provided by the straight line model (partial $F_{1,162} = 176$, $p < 0.001$). Also the higher order cubic model does not improve this predictive power of the quadratic model (partial $F_{1,161} = 0.738$, $p > 0.25$) and is therefore not a suitable model to be used, since it is computationally more tedious. The quadratic regression line is shown in Fig. 1. Fig. 2 shows the residuals plotted against gestational age for the straight line and quadratic models and clearly confirms the superiority of the latter model. These statistical analyses confirm that the rate of rise of hCG is not linear but follows a quadratic curve, that is, it decreases as gestational age increases until a plateau is reached.

In view of this curvilinear relationship, gestational age was divided into three phases (Fig. 1) based on what appeared to be the most appropriate divisions when inspecting the scattergram. Polynomial regression analysis for the best-fitting model was performed for each phase (Table II). In each of these phases, all three models demonstrated an overall regression that was significant and all models did not demonstrate a lack of fit indicating that all were appropriate. However, the higher order models did not improve the predictive power of the straight-line model. These results support the three divisions of gestational age and allow inferences to be made within each phase as hCG now increases in a *linear* fashion with gestational age. With this information, simple linear regression analysis was performed on hCG levels for each patient within each phase. The mean and lower 2 SD were calculated for the slope parameters in each phase (Table III). The mean and lower 2 SD of the doubling times were also calculated for each phase (Table IV) with use of the following formula:

$$\text{Doubling time} = \frac{\log 2}{\text{slope}}$$

Doubling times can be translated into a percentage increase in hCG over time for each phase and thereby provide normal values that may be clinically useful (Table V).

Comment

The application of radioimmunoassay to the detection of hCG has provided a very sensitive and specific test that allows the early detection of pregnancy. As this test is becoming more readily available, it is increasingly being used to determine whether an early pregnancy is advancing normally. This is particularly useful in

Table II. Polynomial regression analysis of early pregnancy data per gestational phase

*Linear regression models**	*F values*		
	Overall regression	*Lack of fit*	*Improved predictive power with use of quadratic vs. straight line*
Phase I† (n = 76)			
Straight line	384 ($p < 0.0001$)	1.53 ($p > 0.25$)	0.19 ($p > 0.25$)
Quadratic	190 ($p < 0.0001$)	0.97 ($p > 0.25$)	
Phase II† (n = 74)			
Straight line	129 ($p < 0.0001$)	0.53 ($p > 0.25$)	1.97 ($p > 0.1$)
Quadratic	66 ($p < 0.0001$)	0.44 ($p > 0.25$)	
Phase III† (n = 21)			
Straight line	5.98 ($p = 0.0244$)	0.67 ($p > 0.25$)	0.17 ($p > 0.25$)
Quadratic	2.95 ($p = 0.078$)	0.77 ($p > 0.25$)	

*Cubic model was not included, since tolerance limits set at 0.0001 had been reached.
†Phase I = Gestational age ≤41 days. Phase II = 41-57 days. Phase III = 57-65 days.

Table III. Straight line slope parameters for each gestational phase

	Phase		
	I	*II*	*III*
Mean slope	0.358	0.146	0.0263
Lower 2 SD	0.272	0.092	0.0213

Table IV. Doubling times for each gestational phase

	Phase		
	I	*II*	*III*
Doubling time	1.94	4.75	26.36
Lower 2 SD	2.55	7.53	32.54

women who are at high risk for recurrent abortion and ectopic pregnancy and also in women who have symptoms of uterine bleeding and/or lower abdominal pain. In these situations, serial hCG determinations are used as prognostic indicators of the status of the pregnancy. To distinguish between normal and ectopic pregnancies, it has been shown that measuring the rate of hCG increase in serum is a more effective method than use of hCG values alone.[7] Several reports have indicated that the serum hCG levels in patients with ectopic pregnancies are much lower than those seen in normal pregnancies.[6,9-11] However, this approach has significant limitations, since often the hCG levels may not be low but the rate of increase may be suboptimal.[12] It would appear that serial hCG determinations would provide more useful information in such cases.

The literature has focused on simple linear regression of log-transformed hCG levels to evaluate early pregnancies. This has resulted in many normal pregnancies being erroneously classified as advancing suboptimally (Daya S, unpublished observations). For example, the straight-line model for the hCG levels in this paper (before subdivision into phases) had a slope of 0.199. The doubling time corresponding to this is 3.48 days. With use of this model, all of the patients who were sampled in phase 2 (gestational age, 41 to 57 days) would be considered as having abnormal hCG increases, since the mean doubling time in this phase was much longer (4.75 days). Similarly, Kadar et al.[12] produced tables showing lower limits of the percentage increase in hCG between test samples drawn several days apart (for example, for a sampling interval of 2 days the percent increase in hCG equals 66%). With use of these tables, many patients sampled in phase 2 in this study again would be considered as having abnormally advancing pregnancies. These interpretations are based on the assumption that hCG increases in early pregnancy at a constant rate. This study clearly shows that the rate of rise of hCG is not constant but is gradually decreasing with advancing pregnancy. The fact that the quadratic regression model provides a better fit for the data than the simple linear regression model supports this observation.

Many of the reports in the literature have used a cohort of patients to provide several samples in order to determine the rate of rise of hCG. This introduces both intrapatient and interpatient variability. The method used in this paper has attempted to reduce

Table V. Percentage increase in hCG over time*

Time interval betwee tests (days)	Phase					
	I (≤41 days)		*II (41-57 days)*		*III (57-65 days)*	
	Increase (%)	*Lower 2 SD*	*Increase (%)*	*Lower 2 SD*	*Increase (%)*	*Lower 2 SD*
1	43	30	17	10	2.7	2
2	103	73	33	20	5.3	4.3
3	193	127	53	33	8.3	6.7
4	320	197	80	43	11	9
5	500	290	107	60	14	11.3
6	757	410	140	73	17	13.7
7	1127	570	177	90	20.3	16

intrapatient variability by use of the slope of hCG increase in each patient to determine the normal values. This allows a more sensitive method of interpreting hCG increases in the pregnant population without having the bias of intrapatient variability.

Division of the gestational age (from day 20 to 66) into three phases has provided a method of evaluating the progress in early pregnancy without violating the principles of regression analysis. The rate of rise is relatively constant in each of these phases. It is important to note that with use of regression analysis, one cannot ignore the gestational age in predicting the rate of rise of hCG. This is especially important in following the cases of women at high risk for spontaneous abortion and ectopic pregnancy so that important prognostic information can be provided. Similarly it may be especially relevant in infertile women who are known to be at high risk of having pathologic pregnancies and in whom gestational age is often known from basal body temperature graphs. In such patients the normal values presented in this paper would be very useful. To determine the minimum acceptable change in hCG required to classify the pregnancy as one that is advancing normally, receiver operating characteristics curve analysis will have to be performed. With use of this method the rate of change in pathologic pregnancies can be compared with normal pregnancies to determine the best "cut-off" that will be clinically useful as a prognostic indicator.

REFERENCES

1. Ascheim S, Zondek B. Hypophysenvorderlappenhormon und Ovarialhormon. Klin Wochenschr 1927;6:1322.
2. Sheehan C. Current status of pregnancy testing. Am J Med Tech 1983;49:485.
3. Vaitukaitis JL, Braunstein GD, Ross GT. A radioimmunoassay which specifically measures human chorionic gonadotropin in the presence of human luteinizing hormone. AM J OBSTET GYNECOL 1972;113:751.
4. Braunstein GD, Rasor J, Adler D, Danzer H, Wade ME. Serum and human chorionic gonadotropin levels throughout normal pregnancy. AM J OBSTET GYNECOL 1976;126:678.
5. Batzer FR, Schlaff S, Goldfarb AF, Corson SL. Serial β-subunit human chorionic gonadotropin doubling time as a prognosticator of pregnancy outcome in an infertile population. Fertil Steril 1981;35:307.
6. Braunstein GD, Karow WG, Gentry WC, Rasor J, Wade ME. First-trimester chorionic gonadotropin measurements as an aid in the diagnosis of early pregnancy disorders. AM J OBSTET GYNECOL 1978;131:25.
7. Kadar N, DeCherney AH, Romero R. Receiver operating characteristic (ROC) curve analysis of the relative efficacy of single and serial chorionic gonadotropin determinations in the early diagnosis of ectopic pregnancy. Fertil Steril 1982;37:542.
8. Pittaway DE, Reish RL, Wentz AC. Doubling times of human chorionic gonadotropin increase in early viable intrauterine pregnancies. AM J OBSTET GYNECOL 1985; 152:299.
9. Kosasa TS, Taymor ML, Goldstein DP, Levesque LA. Use of a radioimmunoassay specific for human chorionic gonadotropin in the diagnosis of early ectopic pregnancy. Obstet Gynecol 1973;42:868.
10. Rasor RL, Braunstein GD. A rapid modification of the beta-hCG radioimmunoassay: use as an aid in the diagnosis of ectopic pregnancy. Obstet Gynecol 1977;50:553.
11. Saxena BB, Landesman R. The use of a radioreceptor assay of human chorionic gonadotropin for the diagnosis and management of ectopic pregnancy. Fertil Steril 1975;26:397.
12. Kadar N, Caldwell BV, Romero R. A method of screening for ectopic pregnancy and its indications. Obstet Gynecol 1981;58:162.

Prevalence of antibody to *Chlamydia trachomatis* in spontaneous abortion and infertility

P. A. Quinn, Ph.D., M. Petric, Ph.D., M. Barkin, M.D., J. Butany, M.D., C. Derzko, M.D., M. Gysler, M.D., K. I. Lie, M.D., A. B. Shewchuck, M.D., J. Shuber, M.D., E. Ryan, M.D., and M. L. Chipman, M.A.

Toronto, Ontario, Canada

A higher prevalence of *Chlamydia trachomatis* antibody occurred in 57.6% of women with recurrent abortion, but not in their male partners, compared to 33.7% of normal pregnant women ($p < 0.01$) and 44.2% of infertile women (NS). The mean titer for the recurrent abortion group was not significantly elevated, compared to controls. Women with blocked fallopian tubes had the highest prevalence of elevated titers ($p < 0.01$) and the highest mean titer ($p < 0.001$). Despite multiple testing, no women or men were chlamydia culture–positive. The lack of isolation among patients with antibody could be due to cryptic infection at a site not amenable to culture or to inhibition by secretory IgA. There could also be nonspecific stimulation of chlamydial antibody caused by other infections such as mycoplasma. The presence, though at a low level, of antibody in culture-negative patients suggests chlamydia may not be directly associated with recurrent abortion but reflect previous exposure to chlamydia or an altered immune system. (Am J Obstet Gynecol 1987;156:291-6.)

Key words: Chlamydia, antibody, recurrent spontaneous abortion, infertility

A number of studies have demonstrated that *Chlamydia trachomatis* plays a prominent role in disorders of the human reproductive system. These include tubal infertility,[1, 2] ectopic pregnancy,[3] and pelvic inflammatory disease.[4] It is the postinflammatory sequelae of the chlamydial infections that are believed to play the major role in the development of the infertile state in the patients.[4] In men these organisms are associated with urethritis, prostatitis, and epididymitis, of which the latter two conditions could affect fertility.[5] Most of these studies have been carried out by examining the serologic status of the populations investigated.

To date there has been little documentation of the role of chlamydial infection in spontaneous abortions. There is evidence, however, that *C. psittaci* is involved in bovine abortion.[6] In the present study the prevalence of antibody to chlamydia is prospectively examined in a major cohort of patients with recurrent spontaneous abortion and compared to normal fertile and infertile patients.

From the Departments of Bacteriology and Virology, The Hospital for Sick Children; Departments of Obstetrics and Gynecology, Toronto General Hospital, Mount Sinai Hospital, St. Joseph's Health Centre, and St. Michael's Hospital; the Department of Pathology, Toronto General Hospital, and the Department of Preventive Medicine and Biostatistics at the University of Toronto.

This project was supported by Health and Welfare Canada Grant No. 6606-2140-53 and Ontario Ministry of Health Grant No. PR-547.

Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.

Reprint requests: Dr. P. A. Quinn, Ph.D., Department of Bacteriology, The Hospital for Sick Children, 555 University Ave., Toronto, Ontario, Canada, M5G 1X8.

Material and methods

Patient populations. Serum samples were obtained from patients enrolled in two prospective studies. The first study examined the role of infection in spontaneous pregnancy loss from 1984 to 1986. The second study examined the role of genital mycoplasmas in infertility between 1976 and 1980. Blood samples were collected on entry to the first study from couples with two documented pregnancy losses including 63 women who enrolled already pregnant, 38 women who were not pregnant, and 55 of their male partners. The control group included 63 normal pregnant women participating as controls in the spontaneous abortion study and 38 nonpregnant women participating as controls in the infertility study. To provide a comparison population in which chlamydia antibody may be elevated, serum samples were assayed on entry from patients enrolled in the infertility study. These patients included 95 women with infertility of known etiology; 25 women who were infertile for no known cause, that is, "normal"; and 116 male partners of these infertile women. The male control group consisted of 36 partners of the normal pregnant women enrolled as controls in the infertility study.

The serum fraction of the blood collected from the above subjects by venipuncture was stored at −20° C until assayed for chlamydial antibody.

Chlamydia antibody assay. The immunofluores-

Table I. Prevalence of chlamydia antibody in women

Status	No. of patients	Any antibody of ≥1:8 dilution		p value	Elevated antibody of ≥1:32 dilution		p value
		n	%		n	%	
Normal controls	101	34	33.7[1]		16	15.8	
Recurrent aborters	66	38	57.6	< 0.01	19	28.8	3.78*
Infertile	120	53	44.2	NS	20	16.7	NS
Any cause	95	44	46.3	NS	19	20.0	NS
No known cause	25	9	36.0	NS	1	4.0	NS

*χ^2 of 3.84 was considered significant; thus a borderline value is indicated.

Table II. Comparison of prevalence of chlamydia antibody among pregnant and nonpregnant women

Status	No. of patients	Any antibody of ≥1:8 dilution		p value	Elevated antibody of ≥1:32 dilution		p value
		n	%		n	%	
Normal controls							
Pregnant	63	17	27.0		10	15.9	
Nonpregnant	38	17	44.8	NS	6	15.8	NS
Recurrent aborters							
Pregnant	34	20	58.8		11	32.3	
Nonpregnant	32	18	56.2	NS	8	25.0	NS

cence microscopy method was used in all assays.[7] The reagents were purchased as a commercial indirect fluorescent antibody kit (Electro-Nucleonics Inc., Columbia, Maryland). The serum samples were first screened at a dilution of 1:8 in phosphate-buffered saline solution (Oxoid Canada, Ltd., Ottawa, Ontario) for the presence of antibody. Those samples manifesting the presence of antibody were then titrated to determine the individual titer. The highest dilution before extinction was taken as the titer of the chlamydial antibody.

Microbiology. Cultures for chlamydia were performed for all couples with recurrent abortions. Cervical and male urethral swabs were taken for culture from the pregnant and nonpregnant couples on entry to the study. Cultures also were made of the endometrial biopsy specimens of the nonpregnant women at the time of entry. Cultures were made of the same male and female specimens at the first prenatal visit and at 20 weeks of gestation for all pregnant couples. *C. trachomatis* was isolated in Hela 229 cells treated with cycloheximide at 2 μg/ml.[8] The specimens were inoculated under centrifugation and monitored at 48 hours by immunofluorescent staining with use of a monoclonal antibody (Syva Diagnostics, Montreal, Quebec).

Data analysis. Comparisons of the prevalence of antibody titers were made with use of the χ^2 test of Fisher's exact test. Mean titers and standard deviations were calculated on the antibody titer converted to $\log_{10}$. The differences in the mean titers were compared by the *t* test.

Results

Prevalence of chlamydia antibody in women with recurrent abortions. Serum samples from a total of 287 women were analyzed for antibody to chlamydia. These were scored for any detectable antibody at a dilution of ≥1:8 and for elevated antibody at a dilution of ≥1:32 as shown in Table I. The latter dilution was interpreted to reflect an appreciable titer of antibody consistent with a relatively recent or severe infection, whereas lower antibody levels were taken to reflect either a milder or more distant infection. The prevalence of any antibody titer (≥1:8) to chlamydia in the normal control women averaged 33.7%. A similar prevalence was seen overall in infertile women. In contrast, the prevalence of any chlamydial antibody in patients with a history of recurrent spontaneous abortion was significantly higher at 57.6% ($p < 0.01$). When the infertile group was subdivided, women with a known cause of pregnancy loss tended to have a higher prevalence than "normal" infertile women. No significant difference was observed in the prevalence of chlamydial antibody between pregnant and nonpregnant recurrent aborters or controls, as shown in Table II.

When the prevalence of elevated antibody titers (≤1:32) was assessed, the recurrent aborters exhibited a trend toward a higher prevalence than the normal

Table III. Prevalence of chlamydia antibody in male partners

Status	No. of patients	Any antibody of ≥1:8 dilution		p value	Elevated antibody of ≥1:32 dilution		p value
		n	%		n	%	
Normal controls	36	17	47.2		4	11.1	
Recurrent aborters	55	23	41.8	NS	14	25.4	NS
Infertile	116	54	46.6	NS	19	16.4	NS
Any cause	91	45	49.5	NS	15	16.5	NS
No known cause	25	9	36.0	NS	4	17.0	NS

Table IV. Correlation of chlamydia antibody to cause of reproductive failure

Status	No. of patients	Elevated antibody of ≥1:32 dilution		p value
		n	%	
Females				
Normal controls	38	6	15.8	
"Normal" infertile	25	1	4.0	NS
Ovulation defect	42	8	19.0	NS
Blocked tubes	7	5	71.4	< 0.01
Adhesions	5	2	40.0	NS
Uterine anomalies	13	0	0.0	NS
Repeat aborters	11	1	9.1	NS
Endometritis	2	0		
Cervical stenosis	2	0		
Trauma	3	1		
Vaginitis	2	1		
Sperm-mucus incompatibility	2	1		
Males				
Normal controls	36	4	11.1	
"Normal" infertile	25	4	17.0	NS
Oligospermia	35	4	11.4	NS
Varicocele	7	1	14.3	NS
Repeat aborters	13	4	30.8	NS
Trauma	3	0		
Blocked tubes	1	0		
Azoospermia	0	0		

controls, though this was not significant ($\chi^2 = 3.78$). These studies therefore suggest that there is a correlation between a history of recurrent spontaneous abortion and the presence of detectable antibody to *C. trachomatis*.

Prevalence of chlamydial antibody in male partners. The prevalence of antibody to chlamydia was next examined in the male partners of the women with a history of recurrent abortion. As shown in Table III, the prevalence of detectable antibody or an elevated antibody response was comparable in the three groups of men.

Isolation of chlamydia trachomatis from couples with recurrent abortions. In all patients in the recurrent abortion study, cultures were made extensively for *C. trachomatis*. In 33 pregnant and 32 nonpregnant couples, repeat cultures were performed on both partners on entry to the study and during gestation. Despite this repeated testing, chlamydia was not isolated from these recurrent aborters. Identical culture conditions in the same laboratory resulted in an isolation rate of 10% to 15% from a population of sexually active adolescent women presenting with a history of vaginal discharge.

Correlation of chlamydia antibody to causes of infertility. A number of studies have suggested that chlamydial infections play a role in subsequent infertility. Accordingly, serum samples from the infertility study patients were analyzed in reference to the documented causes of their reproductive failure. The presence of any detectable antibody was observed in six of seven (85.7%) of the cases with blocked fallopian tubes compared with 17 of 38 (44.8%) of normal controls. As demonstrated in Table IV, there was a significantly increased prevalence of elevated antibody to chlamydia

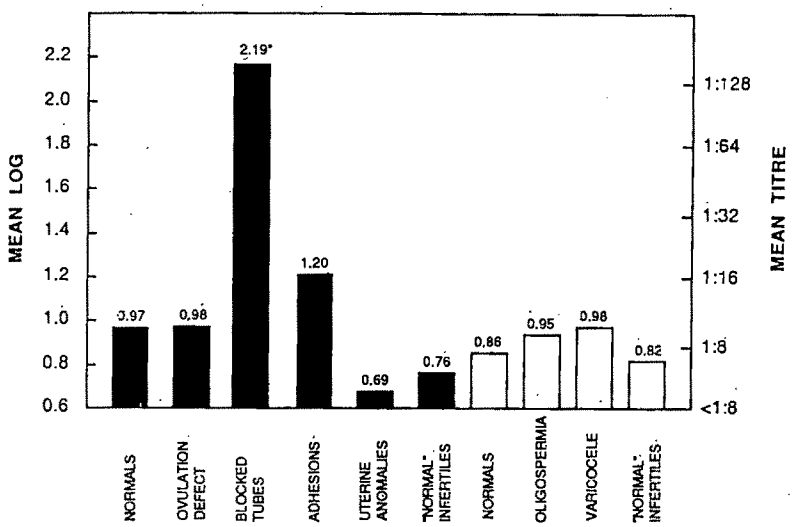


Fig. 1. Mean antibody titers from patients with reproductive failure. *Solid bar,* Women; *open bar,* men. Categories of reproductive failure and controls are indicated. * = $p < 0.01$. Actual mean titers are given over each bar.

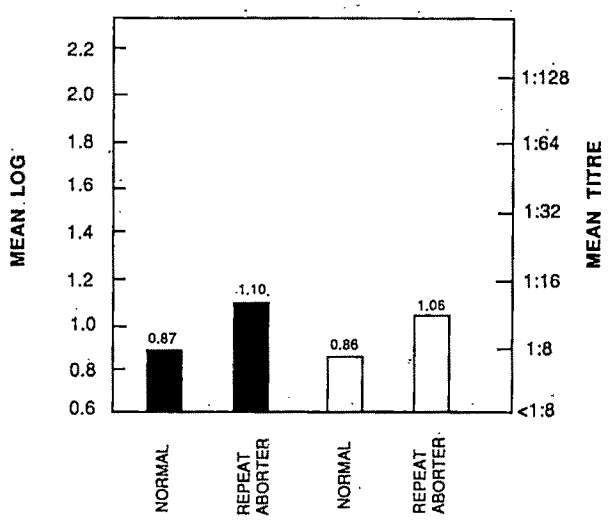


Fig. 2. Mean antibody titers from recurrent abortion patients and normal controls. *Solid bar,* Women; *open bar,* men. Actual mean titers are given over each bar.

in cases with blocked tubes ($p < 0.01$). The second group that exhibited a higher incidence of chlamydial antibody were five women with adhesions, of which three had detectable antibody and two had elevated levels.

Mean antibody titers among couples with reproductive failure and recurrent abortion. To determine whether there was an overall difference in the chlamydial antibody response, the mean titer for each group was compared. The highest mean titer occurred among infertile women, clinically diagnosed as having blocked tubes, as shown in Fig. 1 ($p < 0.01$). Women with adhesions had a slightly higher mean titer. Overall, women and partners with infertility resulting from other defined causes had mean titers comparable to normal controls. When the mean titer of women and partners with a history of recurrent abortion were compared to normal controls, there was no significant difference, as shown in Fig. 2.

Comment

A role for chlamydia infection in recurrent abortion has been proposed but not documented at this time. In our studies of infection in perinatal death, no evidence of chlamydial infection was found in the cases studied.[9] In this study on infection in couples with recurrent spontaneous abortion, patients were examined, by culture and serologic methods, for evidence of chlamydial infection. An increased prevalence of detectable chlamydial antibody was observed in patients with a history of recurrent abortion compared to normal fertile couples. The prevalence of elevated antibody titers was also higher. However, the mean titer was not higher than that of the control group. There was no evidence of an increased prevalence of chlamydial antibody in spouses of recurrent aborters. No difference was observed in the prevalence of chlamydial antibody between pregnant and nonpregnant women. Hence the pregnant state did not itself significantly affect the patients' immune status to chlamydia.

In patients with blocked fallopian tubes, a previously reported sequela of chlamydial infection, both elevated individual titers, and an elevated mean titer, were observed. These latter observations not only confirm the previously reported prevalence of chlamydial antibody in this group[1] but also serve as a positive control on our assay system. The prevalence of low-level chlamydial

antibody of 33.7% in the local normal population is lower than the 71.6% reported elsewhere.[1] This may well be due to the fact that women in our reproductive failure studies are older with a mean age of 28 to 30 years.[9] Evidence of chlamydial infection in these women is less when compared to infection in our adolescents who have a higher rate of isolation of the organism. Further studies are required to determine when women with recurrent abortions actually develop the antibody to chlamydia. In a recent study, residual chlamydial antibody was detected up to 6 years after an episode of pelvic inflammatory disease.[10]

Despite repeated testing, no chlamydia were isolated from this group of couples with recurrent abortion. This suggests that these organisms were either not present at the time of sampling or were present at deeper sites that were not amenable to sampling or their isolation was inhibited by the presence of secretory IgA.[10]

The above results mainly suggest an association of chlamydia antibody and recurrent abortion. There could, however, be other explanations for these antibody responses. A large number of the recurrent aborters are colonized in the genital tract with mycoplasmas, particularly *Ureaplasma urealyticum.* Hence the above two infectious agents may have been acquired simultaneously. The presence of chlamydia antibody could therefore be due to residual antibody from past infection. Since the mean chlamydial antibody titers were not as high in the recurrent aborters as in the patients with blocked tubes, the former patients may have had a more superficial infection. In this study, since no chlamydia were isolated from the recurrent aborters and the mean titer was relatively low, the data suggest that the organisms were not likely to be playing an active role at the time of the study.

Recent evidence strongly suggests that *U. urealyticum* is associated with infection in pregnancy. In women with placentas positive for *U. urealyticum,* there is an association with chorioamnionitis, low birth weight, spontaneous abortion, stillbirth, prematurity, and neonatal death.[9, 11, 12] In cases of perinatal death there is a higher prevalence of the isolation of *U. urealyticum,* an elevated maternal and fetal ureaplasma antibody, and the presence of chorioamnionitis, particularly among cases with no morphologic cause of death.[9, 13]

Since many recurrent abortion patients appear to be infected with *U. urealyticum,* this mycoplasma, like *Mycoplasma pneumoniae,* may nonspecifically alter the patients' immune system.[14] *M. pneumoniae,* the only human mycoplasma infection studied extensively, has been shown to act as a nonspecific polyclonal B cell activator. During episodes of *M. pneumoniae* infection, increases in antibody titers to previous viral infections have been documented and the formation of various autoantibodies described.[14] It is therefore possible that other mycoplasma infections, such as *U. urealyticum,* could nonspecifically stimulate the production of chlamydial antibodies in patients with past chlamydial infections or make them more susceptible to other types of infection.

We have now documented that women with recurrent abortions have elevated antibody titers to *U. urealyticum*[9, 13] and to chlamydia. Recent reports suggest that women with recurrent abortions may have an alteration in their immune system, with the production of antiphospholipid antibodies such as lupus anticoagulant antibody, anti–deoxyribonucleic acid, smooth muscle and cardiolipin antibodies that cross-react in the VDRL antibody test.[15] With *M. pneumoniae* infection, various autoantibodies including cold agglutinins, cold reactive antilymphocyte antibodies, antibodies to brain, lung, smooth muscle, and perhaps most important, to cardiolipin of both IgM and IgG classes have been documented.[14] *M. pneumoniae,*[14] *M. hominis,* and recently *U. urealyticum* have been shown to synthesize phospholipids, and the latter have phospholipases.[16] In *M. pneumoniae,* this phospholipid is distinct from cardiolipin but is reactive with Wassermann-positive sera.[14]

Since the presence of various phospholipid antibodies is being found in recurrent aborters and since ureaplasma infection is associated with intrauterine infection and pregnancy loss in the same population, the two factors may be indirectly associated. It is therefore important that immunologic and infection studies of recurrent abortion patients be coordinated.

In conclusion, elevated antibodies to chlamydia have been detected in a recurrent abortion population. They may not be directly associated with current infection but rather reflect a past infection or alteration of the immune system.

We wish to acknowledge the able technical assistance of S. Mitra and M. Wojtak.

REFERENCES

1. Gump DW, Gibson M, Ashikaga T. Evidence of prior pelvic inflammatory disease and its relationship to *Chlamydia trachomatis* antibody and uterine contraceptive device use in infertile women. AM J OBSTET GYNECOL 1983;146:153.
2. Brunham RC, Maclean IW, Binns B, et al. *Chlamydia trachomatis:* its role in tubal infertility. J Infect Dis 1985; 152:1275.
3. Svensson L, Mardh P-A, Ahlgren M, et al. Ectopic pregnancy and antibodies to *Chlamydia trachomatis.* Fertil Steril 1985;44:313.
4. Mardh P-A. An overview of infectious agents of salpingitis, their biology, and recent methods of detection. AM J OBSTET GYNECOL 1980;138:933.
5. Treharne JD, Forsey T. Chlamydial serology. Br Med Bull 1983;39:194.
6. Storz J, McKercher D, Horwath JA, et al. The isolation of a viral agent from an epizootic bovine abortion. Am Vet Med Assoc J 1960;137:509.
7. Richmond SJ, Caul EO. Fluorescent antibody studies in chlamydial infections. J Clin Microbiol 1975;1:345.

8. Stephens RS, Kuo C-C, Tam MR. Sensitivity of immunofluorescence with monoclonal antibodies for detection of *Chlamydia trachomatis* inclusions in cell culture. J Clin Microbiol 1982;16:4.
9. Quinn PA, Butany J, Chipman M, et al. A prospective study of microbial infection in still births and early neonatal death. AM J OBSTET GYNECOL 1985;151:238.
10. Puolakkainen M, Vesterinen E, Purola E, et al. Persistence of chlamydial antibodies after pelvic inflammatory disease. J Clin Microbiol 1986;23:924.
11. Kundsin RB, Driscoll SG, Monson RR, et al. Association of *Ureaplasma urealyticum* in the placenta with placental morbidity. N Engl J Med 1984;310:641.
12. Embree JE, Krause VW, Embil JA, et al. Placental infection with *Mycoplasma hominis* and *Ureaplasma urealyticum:* clinical correlation. Obstet Gynecol 1980;56:475.
13. Quinn PA. Evidence of an immune response to *Ureaplasma urealyticum* in perinatal morbidity and mortality. Pediatr Infect Dis 1986;5:S282.
14. Biberfeld G. Infection sequelae in autoimmune reactions in *Mycoplasma pneumoniae* infection. In: Razin S, Barile MF, eds. The mycoplasmas. IV. Mycoplasma pathogenicity. New York: Academic Press, 1985:293.
15. Branch DW, Scott JR, Kochenour NK, et al. Obstetric complications associated with lupus anticoagulant. N Engl J Med 1985;313:1322.
16. DeSilva NS, Quinn PA. Endogenous activity of phospholipase A and C in *Ureaplasma urealyticum.* J Clin Microbiol 1986;23:3.

Infertility surgery for pelvic inflammatory disease: Success rates after salpingolysis and salpingostomy

Mark Carey, M.D., and Stan Brown, M.D.
London, Ontario, Canada

Eighty-seven patients with infertility due to pelvic inflammatory disease were retrospectively studied to determine pregnancy rates after infertility surgery. Patients were divided into two groups on the basis of the type of surgical procedure: group 1, lysis of adhesions only (22 patients); group 2, terminal salpingostomy with lysis of adhesions (65 patients). A significantly greater number of patients in group 1 (nine of 22, 41%) than in group 2 (12 of 65, 18%) ($p < 0.05$) achieved intrauterine pregnancy. The incidence of ectopic pregnancy was 23% in group 1 (five of 22) and 9% in group 2 (six of 65) ($p = 0.12$). Almost all pregnancies that were conceived more than 16 months after operation were ectopic gestations. Patients were classified according to severity of disease on the basis of several factors. Although few patients with severe pelvic inflammatory disease conceived after operation, the effect of disease severity on the likelihood of successful operation was not statistically significant. (AM J OBSTET GYNECOL 1987;156:296-300.)

Key words: Pelvic inflammatory disease, infertility surgery, salpingostomy

There are numerous studies in the literature that examine pregnancy rates after tubal reconstructive surgery.[1-5] Many of these studies include patients with a variety of causes of infertility. As well, the accuracy of reported pregnancy rates may be questioned in some studies where patients were not analyzed with respect to the type of surgical procedure. The aim of this study was to examine the success rates of fertility-promoting surgery specifically in patients with pelvic inflammatory disease. Patients were categorized according to the type of surgical procedure, and factors thought to influence the likelihood of a successful operation were studied.

From the Department of Obstetrics and Gynecology, University of Western Ontario.
Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.
Reprint requests: Dr. Stan Brown, University Hospital, 339 Windermere Road, London, Ontario, Canada N6A 5A5.

Material and methods

From January 1978 to April 1985 a retrospective review was performed on all patients in the senior author's practice (S. Brown) who underwent tubal microsurgery. Patients were selected for entry into the study if they fulfilled the following requirements: (1) infertility >1 year's duration, (2) no other significant causes of infertility aside from obstructed fallopian tubes or periadnexal adhesions that were secondary to pelvic inflammatory disease, and (3) follow-up of >18 months' duration. Patients with endometriosis or proximal tubal obstruction were excluded from the study. Patients who required salpingostomy were included only if a terminal salpingostomy was performed.

All patients underwent a complete infertility investigation, which included history, physical examination, documentation of ovulation, evaluation of serum prolactin level, laparoscopy, and hysterosalpingography in

Table I. Patient characteristics

Characteristic	*Group 1: Lysis of adhesions (n = 22)*	*Group 2: Salpingostomy (n = 65)*
Age (yr) (mean ± SD)	28 ± 3.7	28 ± 4.2
Primary infertility	8 (36%)	35 (54%)
Secondary infertility	14 (64%)	30 (46%)
Follow-up (mo) (mean ± SD)	42 ± 17	43 ± 17
Lost to follow-up	1 (4.5%)	6 (9%)
Previous infertility surgery	2 (9%)	12 (18%)
Previous ectopic pregnancy	7 (32%)	9 (14%)
Ovulatory dysfunction (No. given clomiphene citrate)	3	3
Mild hyperprolactinemia	1	0

selected cases; all husbands had a normal semen analysis.

The patients were then divided into two groups: group 1 (22 patients) included those patients with periadnexal adhesions and patent fallopian tubes who required lysis of adhesions only. Patients requiring lysis of fimbrial adhesions were included in this group. Group 2 (65 patients) consisted of patients with distal tubal obstruction who required salpingostomy and lysis of adhesions.

All patients were cared for and operated on by the same surgeon. Operation was performed according to conventional microsurgical techniques.[6] Prophylactic antibiotics were used in all patients and treatment was continued for 10 to 14 days after operation. Steroids and antihistamines were not used. One hundred milliliters of 70% dextran was routinely instilled into the pelvis just before the abdomen was closed. Postoperative hydrotubation was not performed.

Detailed operative reports were available on each patient and an analysis of various factors that might have influenced the outcome of operation was performed. The extent of adhesions were classified as follows: mild, fine filmy adhesions covering less than one third of the surface of the tube and/or ovary; moderate, adhesions covering one third or more of the surface of the tube and/or ovary, with tissue planes evident; severe, thick fibrovascular adhesions covering most of the surface of the tube and/or ovary, with destruction of normal tissue planes making dissection very difficult.

Tubal dilatation was classified according to measurement; the categories being normal, ≤2 cm dilatation, or >2 cm dilatation. The condition of the distal tubal mucosa was graded as follows: good, normal mucosa on examination with magnification; fair, patchy areas of normal and abnormal mucosa; poor to absent, no normal mucosa visible or only an isolated area of normal mucosa present. Patients undergoing unilateral salpingostomy with a patent opposite fallopian tube were excluded from the analysis regarding the above factors.

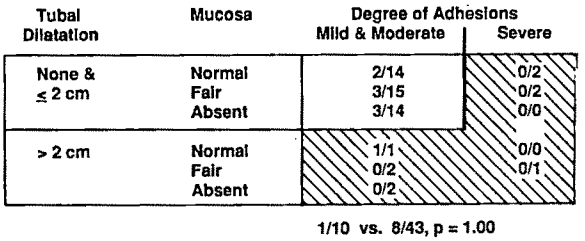


Fig. 1. Factors affecting surgical success classified according to the side of least disease. (Values are number of pregnancies/ number of operations.)

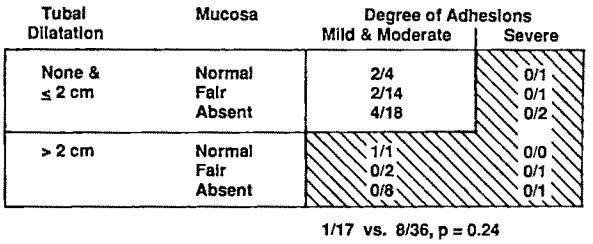


Fig. 2. Factors affecting surgical success classified according to the side of most disease. (Values are number of pregnancies/ number of operations.)

Pregnancies were counted as such only if there was documented evidence of intrauterine or extrauterine pregnancy. This included ultrasound examination in an early intrauterine pregnancy or pathologic examination of the products of conception in cases of spontaneous abortion or ectopic pregnancy. Intrauterine pregnancies were counted only once per patient. Follow-up was maintained for at least 40 months in those patients who did not conceive. Statistical analysis was performed with the use of χ^2 analysis with continuity correction and Fisher's exact test when appropriate. Continuous data were analyzed with the use of Student's t test.

Results

The characteristics of the 87 patients included in the study are shown in Table I. There was no significant difference between groups 1 and 2 with respect to mean ages or the incidence of primary or secondary infertility. When group 1 and group 2 were examined separately, there was no significant difference in age or previous fertility status when those patients who had intrauterine pregnancies were compared with those who did not. Three patients in each group had evidence of ovulatory dysfunction, which was successfully treated with clomiphene. One woman in group 1 had mild hyperprolactinemia, which responded well to bromocriptine.

The overall pregnancy rates and the mean time intervals from operation to pregnancy are presented in Table II. In group 1 (lysis group with lysis of adhe-

Table II. Pregnancy rate and operation-to-pregnancy interval after reconstructive surgery for pelvic inflammatory disease

Group	Total No. of patients	Intrauterine pregnancy		Ectopic pregnancy		Mean operation-to-pregnancy interval (mo)	
		No.	%	No.	%	Intrauterine	Ectopic
Group 1	22	9	41*	5	23†	8 ± 3.2	24 ± 17
Group 2	65	12	18*	6	9†	11 ± 6.9	37 ± 32
Total	87	21	24	11	13	9 ± 5.7*	31 ± 26*

*$p < 0.05$.
†$p = 0.12$

Table III. Lysis of adhesions: Pregnancy outcome versus degree of adhesions

Degree of adhesions	Total patients		Intrauterine pregnancy		Ectopic pregnancy	
	No.	%	No.	%	No.	%
Mild	4	18	3	75	1	25
Moderate	15	68	5	33	3	20
Severe	3	14	1	33	2	67

Table IV. Surgical procedure for pelvic inflammatory disease: Salpingostomy (group 2)

	Total patients		Intrauterine pregnancy		Ectopic pregnancy	
	No.	%	No.	%	No.	%
Bilateral salpingostomy	43	66	8	19*	4	9
Unilateral salpingostomy (opposite tube patent)	12	18	3	25*	2	16
Unilateral salpingostomy (opposite tube absent)	10	15	1	10*	0	0

*p = NS.

Table V. Pregnancy outcome in patients with previous infertility surgery, or ectopic pregnancy

	Total patients		Intrauterine pregnancy		Ectopic pregnancy	
	No.	%	No.	%	No.	%
Previous ectopic pregnancy	16		4	25	4	25
Previous infertility surgery	16		2	12.5	2	12.5

sions), nine patients (41%) had a documented intrauterine pregnancy, and five patients (23%) had tubal ectopic gestations. In group 2 (salpingostomy group) 12 patients (18%) achieved intrauterine pregnancy, while six patients (9%) had tubal pregnancies. The differences between intrauterine pregnancy rates in groups 1 and 2 were significant ($p < 0.05$), while the differences in ectopic pregnancy rates were not ($p = 0.12$). The mean time interval from operation to pregnancy was significantly longer in patients with ectopic gestations than in those with intrauterine pregnancies ($p < 0.05$).

Group 1 was further subdivided according to the severity of adhesions (Table III). There was a trend toward a higher chance of intrauterine pregnancy in the group who had lysis of mild adhesions. This trend, however, was not statistically significant.

Table IV summarizes the surgical procedures in group 2. Forty-three patients underwent bilateral salpingostomy and eight patients (19%) had subsequent intrauterine pregnancies. It should be noted that all patients in group 2 had some form of periadnexal adhesiolysis. Twenty-five percent of the 12 patients who underwent unilateral salpingostomy with the opposite

tube patent achieved intrauterine pregnancy, while only one of 10 patients successfully conceived in the group that had a unilateral salpingostomy with the opposite tube absent. None of the differences in the pregnancy rates between the three groups illustrated in Table IV were statistically significant.

Figs. 1 and 2 summarize the pregnancy rates in group 2 (65 patients) according to the various factors analyzed. Patients who had a unilateral salpingostomy and patent opposite fallopian tubes (12 patients) were excluded from this analysis. In many cases it was apparent that the degree of tubal disease differed between the right and left sides in the patients who underwent bilateral salpingostomy. For this reason, the analysis of the various factors that might influence pregnancy rates was done in two ways. In Fig. 1 patients were categorized according to the side of least disease, whereas in Fig. 2 patients were categorized according to the side with the most disease.

In both Figs. 1 and 2 patients with severe adhesions or tubal dilatation >2 cm were unlikely to achieve intrauterine pregnancy after operation. The condition of the tubal mucosa did not seem to alter pregnancy outcome but the numbers are too small for meaningful statistical analysis. Most pregnancies occurred in the mild to moderate dilatation-adhesion categories, and therefore these categories were grouped together in Figs. 1 and 2 for simplification. Using either classification system, we were unable to prove that there was a significant difference in the proportion of pregnancies between the severe dilatation-adhesion group and the mild to moderate dilatation-adhesion group, as illustrated by comparison of the proportion of pregnancies in the *shaded* versus the *unshaded* areas in Figs. 1 and 2.

Pregnancy outcome was also examined in patients who had previous infertility surgery (lysis of adhesions with or without salpingostomy) and in those who required a previous operation for an ectopic pregnancy. Groups 1 and 2 were combined because of the small number of patients (Table V). The intrauterine pregnancy rates and ectopic pregnancy rates are outlined in Table V.

Comment

Jansen[7] has previously reported that patients with patent fallopian tubes have a significantly better chance of conceiving after fertility surgery than patients with obstructed tubes. In his study, 54% of patients who underwent salpingolysis conceived whereas only 19% of patients with bilateral tubal obstruction became pregnant after operation. This study reports very similar figures, with intrauterine pregnancy rates of 41% in the group with lysis of adhesions (group 1) and 18% in the group requiring salpingostomy (group 2). Jansen was also able to demonstrate a significant difference in pregnancy rates between patients with unilateral versus those with bilateral tubal obstruction (43% versus 19%). In this study the improved pregnancy rates found in the patients with unilateral tubal obstruction (25%) were not significantly different from those of patients with bilateral obstruction (19%).

Ectopic pregnancy was a significant cause of morbidity in this study. There was a 23% incidence of ectopic pregnancy in group 1 and a 9% incidence in group 2. Patients with previous infertility surgery or ectopic pregnancy, although few in number, were also at risk for ectopic gestations. An interesting finding was the lengthened mean time interval from operation to pregnancy in patients with ectopic pregnancies. There was only one intrauterine pregnancy conceived more than 16 months from the time of operation. With a follow-up period of at least 40 months for all patients who did not conceive, it is reasonable to conclude that there is a high risk of ectopic pregnancy in patients who conceive more than 16 months from the time of operation.

The outcome of those patients undergoing bilateral salpingostomy was disappointing. Caspi et al.[2] have reported similar results, with intrauterine pregnancy rates of 20% and ectopic pregnancy rates of 9% in patients after operation for bilateral tubal occlusion. Gomel[8] has reported term pregnancy rates as high as 30% in 89 patients requiring salpingostomy with a 9% ectopic gestation rate.

In patients requiring salpingostomy we were not able to demonstrate a significant effect of the severity of disease on pregnancy outcome. Previous studies by Verhoeven et al.[5] and Rock et al.[9] were able to identify a group of patients with "mild disease" whose chances for pregnancy were significantly better than those of patients with more severe disease. Pregnancy rates for "mild disease" varied from 30% to 40% in Verhoeven's study and were approximately 80% in Rock's study. Pregnancy rates dropped dramatically to ≤10% in patients with severe disease. We were unable to identify such a favorable group of patients. This difference may be related to inherent differences in the patient population and smaller sample sizes, or it may be the result of the effect of our classification system. The effect of classifying patients according to the side of least and most disease, as shown in Figs. 1 and 2, illustrates the subjective nature of classification shemes. Differences in classifying patients may result in marked differences in outcome, rendering comparisons between studies difficult. In the present study, classifying patients according to severity of disease on the basis of the side most diseased best identified those patients not likely to benefit from operation. It appears that there may be a group of patients with severe pelvic inflammatory disease who do not have a reason-

able chance for a successful operation. However, a larger sample size would be needed to prove this statistically.

The management of patients with infertility secondary to pelvic inflammatory disease should be individualized. Patients with patent fallopian tubes are better candidates for operation than those with obstructed tubes. In many patients in vitro fertilization may be a reasonable alternative to operation; its use depends on the in vitro fertilization program availability, the cost, and the patients' needs.

REFERENCES

1. Betz G, Engel T, Penney L. Tuboplasty—comparison of methodology. Fertil Steril 1980;34:534.
2. Caspi E, Halperin Y, Bukovsky I. The importance of peri-adnexal adhesions in tubal reconstructive surgery for infertility. Fertil Steril 1979;31:296.
3. Fayez JA, Suliman SO. Infertility surgery of the oviduct: comparison between macrosurgery with microsurgery. Fertil Steril 1982;37:73.
4. Larsson B. Late results of salpingostomy combined with salpingolysis and ovariolysis by electromicrosurgery in 54 women. Fertil Steril 1982;37:156.
5. Verhoeven HC, Berry H, Frantzen L, et al. Surgical treatment for distal tubal occlusion. J Reprod Med 1983;28:293.
6. Gomel V. Microsurgery in female infertility. Boston: Little, Brown. 1983.
7. Jansen RP. Surgery—pregnancy time intervals after salpingolysis, unilateral salpingostomy and bilateral salpingostomy. Fertil 1980;34:222.
8. Gomel V. Surgical correction of sequelae of pelvic inflammatory disease. J Reprod Med 1983;28:718.
9. Rock JA, Katayama KP, Martin EJ, et al. Factors influencing the success of salpingostomy techniques for distal fimbrial obstruction. Obstet Gynecol 1978;52:591.

Temporal variation in rates of cesarean section for dystocia: Does "convenience" play a role?

William Fraser, M.D., Robert H. Usher, M.D., Frances H. McLean, B.Sc.N., Carol Bossenberry, B.N., Mary Ellen Thomson, Ph.D., Michael S. Kramer, M.D., L. Paul Smith, M.D., and Hugh Power, M.D.

Montreal, Quebec, Canada

The purpose of this study is to test the hypothesis that convenience for the physician plays a role in the rate of cesarean section performed because of dystocia. Three time periods were defined (night, 12 midnight to 7:59 AM; day, 8 AM to 5:59 PM; evening, 6 PM to 11:59 PM) based on the work commitments and daily routines of the obstetrician. Rates of cesarean section for dystocia were determined for each of the three time periods. An evening peak in the cesarean section rate is partially but not entirely explained by an evening increase in the proportion of patients in prolonged labor. When patients were stratified according to labor duration (<12, 12 to 15, and >16 hours), a persistent evening excess in the rate of cesarean section for dystocia was observed for patients whose labor duration was <16 hours. Although this is interpreted as being consistent with the hypothesis of physician convenience, the magnitude of this effect on the overall rate of cesarean section for dystocia is small. (AM J OBSTET GYNECOL 1987; 156:300-4.)

Key words: Cesarean section, dystocia, temporal variation, obstetric convenience

The rapid rise in the rate of cesarean section has come under increasing scrutiny in recent years.[1,2] Performance of cesarean section because of dystocia is of particular concern. Dystocia is the most common indication for primary cesarean section and provides the greatest room for diagnostic discretion.

To what degree does convenience for the physician influence the rate of cesarean section for dystocia? We hypothesized that if this factor is important there should be a regular temporal variation in cesarean section rates in accord with the relative availability of the obstetrician to the delivery room. Cesarean sections for dystocia would be expected to occur less frequently during the nighttime hours of sleep and the daytime period of scheduled office hours and of surgical and teaching commitments. Evening hours would be expected to have the highest incidence, both because of

From the Departments of Obstetrics and Gynecology, Pediatrics, and Epidemiology and Biostatistics, McGill University Faculty of Medicine, and the Royal Victoria Hospital.
Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.
Reprint requests: Dr. Robert H. Usher, Department of Neonatology, Women's Pavillion, Room F 550, Royal Victoria Hospital, 687 Pine Ave. West, Montreal, Quebec, Canada H3A 1A1.

Table I. Rate of cesarean section for dystocia by day of week

Day	Total No. of deliveries	Cesarean section		
		No.	%	
Monday	629	31	4.93	
Tuesday	638	32	5.02	
Wednesday	608	30	4.93	4.76*
Thursday	600	24	4.00	
Friday	592	29	4.89	
Saturday	563	21	3.73	3.78*
Sunday	602	23	3.82	

*NS; $\chi^2 = 2.17$.

Table II. Incidence of cesarean section for dystocia per 100 deliveries

	Total No. of deliveries	Cesarean section	
		n	%
Weekday			
Night	740	33	4.5
Day	1541	60	3.9
Evening	786	55	7.0
Total	3067	148	$p < 0.01$*
Weekend			
Night	284	7	2.5
Day	604	20	3.3
Evening	277	15	5.4
Total	1165	42	NS†

*$\chi^2 = 11.2$.
†$\chi^2 = 3.82$.

fewer conflicting obligations and in order to terminate desultory labors before the normal hours of sleep. Lower rates might also be expected on weekends when one obstetrician is "on call" for his group and does not have conflicting work obligations.

The present study was designed to test the hypothesis that physician convenience plays a role in the decision to perform a cesarean section for dystocia. The practice setting studied is similar to most obstetric situations in private practice. The role of variables other than physician convenience in producing differential cesarean section rates for dystocia by time of day was also explored. An unexpected confounding factor, temporal variation in the proportion of patients in prolonged labor, was discovered. This required careful control in order to attempt to isolate the contribution of physician convenience.

Method

The study was carried out at the Royal Victoria Hospital, a tertiary-care teaching hospital of McGill University in which approximately 3500 women are delivered each year. Deliveries are performed by obstetrician-gynecologists, most of whom perform between 100 and 200 deliveries each year. More than 95% of women who are delivered receive their antenatal care in the private offices of attending staff members. In general, daytime coverage is provided by each obstetrician for his or her own patients, while night and weekend coverage is organized in groups with one physician covering for three to six group members. The obstetrician on call usually comes to the hospital from outside for each delivery. Though deliveries are usually also attended by the house staff, an attending staff obstetrician is almost always present at the time of delivery. Remuneration for all deliveries is from the provincially funded health insurance program. There is no differential tariff for procedures by time of day, and there is little difference between the fee for cesarean section and vaginal delivery.

The study population was selected to focus on the category of patients principally at risk for dystocia: nulliparous women with singleton fetuses in cephalic presentation at term (gestational age >37 weeks). Induced labors were excluded, since inductions are generally initiated early in the day and are followed by a higher incidence of cesarean section for dystocia than are spontaneous labors.[3]

All patients presenting in spontaneous labor on admission to the hospital between January 1978 and April 1984 and meeting the above criteria were included in the study (n = 4232). Data were obtained from the computerized McGill Obstetric and Neonatal Data Base.[4,5] The indication for cesarean section was the one recorded by the obstetric team at the time of delivery. For the purposes of the present study, cesarean sections performed either for failure to progress or for cephalopelvic disproportion were included under the category of dystocia. When fetal distress was recorded as an associated indication along with dystocia, such cesarean sections were considered to have been performed for dystocia.

Only those cesarean sections performed during the first stage of labor were included. Second-stage dystocia has more precisely defined limits and involves a different range of management options than those in the first stage.

Labor onset was defined as time of admission to hospital in established labor, which is an objective and verifiable point of reference for women in labor of spontaneous origin. Women admitted, with or without spontaneous rupture of membranes, whose labor began after admission to hospital were excluded.

Diurnal variation in the incidence of cesarean section for dystocia was analyzed in three time periods chosen a priori: day (8 AM to 5:59 PM), evening (6 PM to 11:59 PM), and night (12 midnight to 7:59 AM).

The rate of cesarean section for dystocia by time of day was expressed in each of two ways: (1) as a pro-

Table III. Hourly risk of cesarean section for dystocia

	Patient-hours of labor	*Cesarean section*	
		n	%*
Night	11,011	40	0.36
Day	17,235	80	0.46
Evening	6,181	70	1.13

*$p < 0.001$; $\chi^2 = 54.2$.

Table IV. Incidence of prolonged labor by time of day*

Time of day	*Mean No. of patients*	*% with labor ≥12 hr*	*% with labor ≥16 hr*
Night	1294	9.9	2.9
Day	1569	12.6	3.5
Evening	884	18.0	6.9

*Mean number of laboring patients per hour for the time period, and mean proportion of laboring patients per hour who were in prolonged labor.

portion of the total deliveries during the time period and (2) as an hourly rate per 100 patient-hours of labor. This latter rate (number of cesarean sections for dystocia per number of patients in labor duing each hour of the day) was considered the more appropriate, because the patients at risk of requiring a cesarean section because of dystocia are those in labor rather than those delivering at a particular hour. Since cesarean sections for dystocia occur much more frequently in patients with prolonged labor and since there was a diurnal variation in incidence of prolonged labor, the rate of cesarean sections for dystocia was also expressed per number of patient-hours of labor of "normal" duration (<12 hours), "longer than average" (12 to 15 hours), and "very prolonged" (>16 hours) at each time of day. The rates of cesarean section for dystocia on weekdays (Mondays to Fridays) and weekends (Saturdays and Sundays) were also determined separately. Statistical calculations were performed by the χ^2 test for proportions and by one-way analysis of variance for differences in means.

Results

Table I expresses cesarean section deliveries as a percent of total deliveries by day of week. Although there is a trend toward fewer cesarean sections for dystocia on weekends than on weekdays (3.78% versus 4.76%), this trend does not achieve statistical significance.

On weekdays the rate of cesarean deliveries varied markedly by time of day, with a significantly higher rate occurring in the evening ($p < 0.01$). A similar trend was seen on weekends, but this did not achieve statistical significance (Table II).

When expressed as 100 patient-hours of labor, cesarean section rates for the laboring population were more than twice as high in the evening as during the day or night ($p < 0.001$) (Table III). For these and subsequent calculations, weekday and weekend data were combined.

Table IV shows the proportion of patients in prolonged labor by time of day. It indicates that during the evening the proportion of patients who were in prolonged labor was twice as great as during the night. Thus the observed evening peak in cesarean sections may be at least partially explained by an evening peak in prolonged labors.

It remained to be determined whether, for patients of similar labor duration, there was temporal variation in the rate of cesarean section for dystocia. To control for variation by time of day in incidence of prolonged labor, laboring patients were stratified in the manner previously described: those with normal labor duration (<12 hours), longer-than-average labor (12 to 15 hours), and very prolonged labor (>16 hours). Table V expresses cesarean section rates per 100 patient hours of labor for the three groups. For patients in very prolonged labor the risk of cesarean section for dystocia did not vary by time of day. For patients with shorter labors, however, the risk of cesarean section varied significantly by time of day, with peak rates occurring in the evening.

Table VI shows mean cervical dilatation at the time of cesarean section and mean labor duration for patients delivered by cesarean section by time of day. Neither cervical dilatation nor duration of labor before cesarean section was found to vary significantly among the three time periods.

Table VII explores other potentially confounding variables that could influence the frequency of cesarean section for dystocia: maternal age, maternal height, maternal weight, and fetal weight. None was found to differ among populations delivered at different times of the day.

Comment

The present study setting is typical of private obstetric practice in North America, in which hospital deliveries are performed by obstetrician-gynecologists whose offices are usually located outside the hospital. From the standpoint of the obstetrician practicing in this setting, there are more and less convenient times to perform cesarean sections for dystocia.

The study began with the stated assumption that if temporal variation in rates of cesarean section for dystocia were demonstrable, this variation might reasonably be attributed to physician convenience. In the course of the study, however, it became evident that this assumption required qualification. The proportion of patients in prolonged labor, which is closely linked

Table V. Hourly risk of cesarean section after differing lengths of labor

	Labor <12 hr			*Labor 12-15 hr*			*Labor ≥16 hr*		
	Patient hours	*Cesarean section*		*Patient hours*	*Cesarean section*		*Patient hours*	*Cesarean section*	
		n	%*		*n*	%†		*n*	%‡
Night	10,358	16	0.15	417	11	2.6	236	13	5.5
Day	15,693	34	0.22	1117	21	1.9	425	25	5.9
Evening	5,307	25	0.47	556	26	4.6	318	19	6.0

*$p < 0.01$; $\chi^2 = 15.5$.
†$p < 0.01$; $\chi^2 = 10.0$.
‡NS; $\chi^2 = 0.056$.

Table VI. Cervical dilatation and duration of labor before cesarean section for dystocia

	Cervical dilatation (cm)			*Duration of labor (hr)*		
	n	*Mean*	*SD*	*n*	*Mean*	*SD*
Night	38	5.4	2.5	40	13.1	7.8
Day	76	4.5	2.4	80	13.0	6.4
Evening	66	4.5	2.3	70	13.4	5.6
Total	180			190		

Table VII. Characteristics of cases according to different times of delivery

	Maternal age (yr)			*Maternal height (cm)*			*Maternal predelivery weight (kg)*			*Infant birth weight (gm)*		
	n	*Mean*	*SD*	*n*	*Mean*	*SD*	*n*	*Mean*	*SD*	*n*	*Mean*	*SD*
Night	40	27.1	5.0	23	158.7	6.6	25	71.6	11.3	40	3532	474
Day	80	27.0	4.4	50	158.9	6.9	42	72.8	12.0	80	3548	440
Evening	70	27.6	4.7	52	159.6	6.8	48	70.7	10.7	70	3555	479

to rates of cesarean section for dystocia, varied considerably by time of day. This variation resulted from an unexpected diurnal variation in time of onset of labor. Admission of patients in spontaneous labor occurred more than twice as frequently during the night hours as during the day (Fraser W, Usher R. Diurnal variation in onset of labour. Submitted for publication). This resulted in a sinusoidal admission curve similar to that described by Shettles[6] for the time of labor onset. The evening period, falling 12 to 18 hours after the nighttime peak of admissions, had the highest proportion of patients in prolonged labor.

It then became necessary to modify the question in the following manner: Do patients who have been in labor for similar lengths of time differ in their risk of cesarean section for dystocia according to the time of day? Patients were stratified by number of hours in labor in a manner which, although not providing complete standardization, should have eliminated most of the difference in mean labor duration between subgroups. After controlling in this manner for the uneven distribution of women in prolonged labor, there remained a persistent excess in cesarean sections for dystocia during the evening hours, except for patients in very prolonged labor for whom the rate of cesarean section was similar at all times of day.

If the decision to perform cesarean section for dystocia was being taken at a lower threshold during the evening, shorter labors and lesser cervical dilatations at the time of cesarean section might be expected. Such differences were not observed. How does one account for this negative finding? It seems that cesarean sections for dystocia were performed on the same category of laboring patient (cervix dilated approximately 5 cm and labor duration approximately 13 hours) regardless of time of day. However, more of these patients were selected for cesarean section during the evening hours.

Are there factors other than physician convenience that could vary by time of day to influence cesarean section rates? Patient tolerance to prolonged labor may be decreased in the evening as a result of fatigue, and this could influence decision making. Availability of nursing staff, house staff, anesthesia staff, and operating room time vary by time of day in some hospitals. In the present study such personnel were in, or immediately available to, the labour and delivery suite around the clock, and a special operating room for

primary cesarean sections was situated within the labour and delivery area.

There have been previous attempts to examine the question of temporal variation in cesarean section rates. Phillips et al.[7] examined cesarean section rates by day of week. His findings in a comparison of weekend and weekday rates were consistent with our own. However, inductions were not excluded from his study population. Evans et al.[8] classified cesarean sections into "acute" and "nonacute" and found fewer nonacute cesarean sections were performed at night than at other times of day. Temporal variation in the proportion of patients in prolonged labor was not considered.

In the present study, temporal variation in rates of cesarean section for patients of similar labour duration, though demonstrable, had a relatively small impact on the overall rate of cesarean section for dystocia. Although this evening peak is consistent with the hypothesis of physician convenience, elimination of this "excess" number would decrease the overall rate of cesarean sections for dystocia by only 15%. Physician convenience does not appear to be the major factor in the recent marked increase in rates of cesarean section for dystocia.

It appears, however, that there are several aspects of labor management in which the timing of physician intervention may have an important effect on rates of cesarean section for dystocia. As previously mentioned, elective inductions are associated with an increased rate.[5] Duff et al.[9] have shown that, in the patient at term with spontaneous rupture of the membranes, expectant management can significantly reduce rates of cesarean section for dystocia below rates that occur when such patients are induced. Further studies are required to determine the impact of artificial rupture of membranes, oxytocin stimulation (including its use in women whose labors are not well established), and continuous epidural analgesia on the progress of labor and rates of cesarean section for dystocia.

REFERENCES

1. Rosen MG, Alper MH, Bloomfield R, Cefalo RC, Field T. NIH Consensus Development Task Force statement on cesarean childbirth. AM J OBSTET GYNECOL 1981;139:901.
2. Minkoff HL, Schwarz RH. The rising cesarean section rate: can it safely be reversed? Obstet Gynecol 56:135, 1980.
3. Smith LP, Nagourney BA, McLean FH, Usher RH. Hazards and benefits of elective induction of labor. AM J OBSTET GYNECOL 1984;148:579.
4. Funnell WRJ, Hosios AJ, Kearney RE. IDA: an interactive data analysis system for clinical and experimental research. Comp Biol Med 1980;10:41.
5. Smith LP, deLeon A, Funnell WRJ, Lalonde AB, McLean FH, Usher RH. A research-oriented system for McGill Obstetrical and Neonatal Data (MOND). Acta Obstet Gynecol Scand [Suppl] 1982;109:49.
6. Shettles LB. Hourly variation in onset of labor and rupture of membranes. AM J OBSTET GYNECOL 1960;79:177.
7. Phillips RN, Thornton J, Gleicher N. Physician bias in cesarean section. JAMA 1982;248:1082.
8. Evans MI, Richardson DA, Sholl JS, Johnson BA. Cesarean section assessment of the convenience factor. J Reprod Med 1984;29:670.
9. Duff P, Huff RW, Gibbs RS. Management of premature rupture of membranes and unfavorable cervix in term pregnancy. Obstet Gynecol 1984;63:697.

HOW TO SET UP AN IVF PROGRAM

"The Team Approach to In-Vitro Fertilization"

■ **June 10-14, 1987**
Harrah's Convention Center
Reno, Nevada

At this very special meeting, you can learn about planning, designing and establishing an IVF program.

This Serono Symposia, USA co-sponsored workshop will help teams of physicians, embryologists, nurse coordinators and psychologists develop a detailed approach to IVF.

Experts from three successful IVF programs will share their experiences through lectures and hands-on laboratory workshops and will lead general discussion sessions.

Plus, marketing professionals will discuss public relations and promotional strategies for IVF programs.

If you're interested in an IVF program, this may well be the most worthwhile meeting you can attend.

■ **Faculty**
Northern Nevada Fertility Clinic

PHYSICIANS:
Geoffrey Sher, M.D., Chairman
Harold Chotiner, M.D.
Victor Knutzen, M.D.
EMBRYOLOGISTS:
Patty Julian, M.S.
Clifford Stratton, Ph.D.
Linda Vaught, Ph.D.
William Vaught, Ph.D.
NURSE COORDINATOR:
Virginia Marriage, R.N., M.N.
PSYCHOLOGIST:
Jack Mayville, Ph.D.

■ **Guest Faculty**

PHYSICIANS:
Ricardo Asch, M.D.
Brian Cohen, M.D.
Gary Hodgen, Ph.D.
Richard Marrs, M.D.
NURSE COORDINATOR:
Catherine Garner, R.N.C., M.S.N., M.P.A.
MARKETING PROFESSIONALS:
Peter Necarsulmer, B.A.
Susan Thurman, B.A.
HEALTH CARE COUNSEL:
William Prezant, Esq.

■ **To register for this unique meeting—"The Team Approach to In-Vitro Fertilization"—contact: Serono Symposia, USA, toll-free at 800/225-5185. In Massachusetts, 617/963-8154.**

Registration Fee:

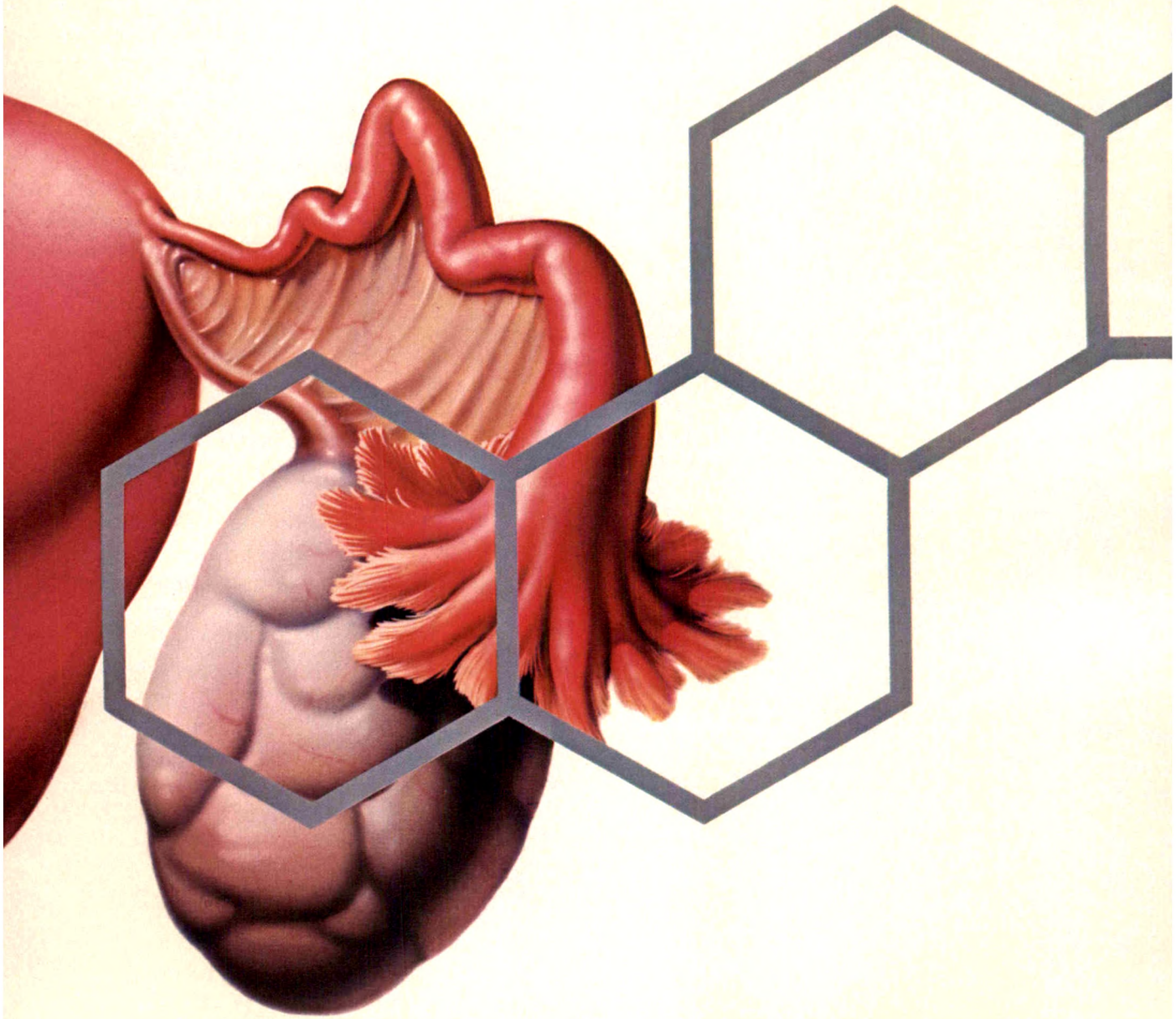
Before March 1	$400 for physicians $200 for support personnel
After March 1	$450 for physicians $250 for support personnel

To reserve special hotel accommodations, call Harrah's directly at 800/648-3773. And mention the Serono Symposia, USA IVF Workshop.

Serono Symposia, USA
280 Pond Street
Randolph, MA 02368

THE RIGHT CHEMISTRY

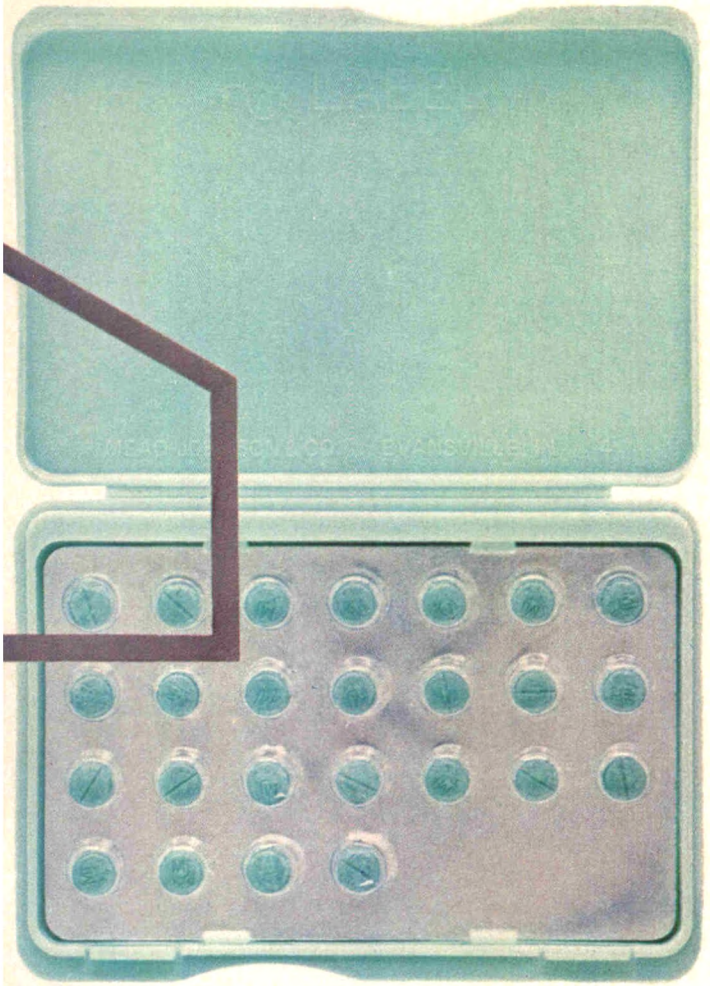
THE RIGHT REPLACEMENT FOR 17β-ESTRADIOL—17β-ESTRADIOL



© 1986 Mead Johnson & Company • Evansville, Indiana 47721 USA MJL 5-4169

THE RIGHT REGIMEN

THE FIRST 25 DAYS OF THE MONTH— EVERY MONTH



ESTRACE is 17β-estradiol—just like the estrogen she made before menopause. Like the natural hormone, it follows a simple, well-known metabolic pathway, and it provides unsurpassed relief of postmenopausal vasomotor symptoms.

Micronized for good oral absorption, ESTRACE produces prompt, predictable serum levels of estradiol and estrone. Between cycles, ESTRACE is rapidly eliminated, providing estrogen replacement therapy that closely follows the premenopausal pattern . . . *for true cyclic therapy.*

Now it's in the ESTRACE Cycle Pak—an easy-to-carry compact, designed to deliver a starting dose of 2 mg in the preferred 25-day regimen. Cycle Paks delivering 1 mg daily are also available, if needed.

Mead Johnson LABORATORIES

For Brief Summary, please see following page.

ONLY NEW ESTRACE® (ESTRADIOL) TABLETS 1 mg and 2 mg CYCLE PAK HAS BOTH

ESTRACE® (estradiol)

TABLETS, 1 mg and 2 mg VAGINAL CREAM, 0.01%

Warning

1. **ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.** Three independent case control studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade. The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. **ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.** The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1000 exposures. Furthermore, a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb reduction defects. One case control study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed *in utero* to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. If ESTRACE® (estradiol) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation.

Indications: *Tablets:* Moderate to severe *vasomotor* symptoms associated with the menopause (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.); atrophic vaginitis; kraurosis vulvae; female hypogonadism; female castration; primary ovarian failure; breast cancer (for palliation only) in appropriately selected women and men with metastatic disease; prostatic carcinoma—palliative therapy of advanced disease. *Vaginal Cream:* Atrophic vaginitis and kraurosis vulvae. ESTRACE® HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

Contraindications: Known or suspected cancer of the breast, except in appropriately selected patients being treated for metastatic disease; known or suspected estrogen-dependent neoplasia; known or suspected pregnancy (See Boxed Warning); undiagnosed abnormal genital bleeding; active thrombophlebitis or thromboembolic disorders; a past history of thrombophlebitis, thrombosis or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

Warnings: Estrogens increase the risk of carcinoma of the endometrium (See Boxed Warning). Prescribe cautiously in women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms. A 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease has been reported in women receiving postmenopausal estrogens. There is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders or (except in treatment of malignancy) with a history of such disorders in association with estrogen use; they should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed. Large doses (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis; when estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptives should be considered a clear risk. Hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Increased blood pressure occurs with use of estrogens in the menopause, and blood pressure should be monitored, especially with high doses. Diabetic patients should be carefully observed for decreased glucose tolerance. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases; if this occurs, the drug should be stopped and appropriate measures taken to reduce the serum calcium level.

Precautions: *General*—A complete medical and family history should be taken prior to initiation of therapy. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination. Conditions influenced by fluid retention, such as epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc, may develop. Patients with a history of depression should be carefully observed. Pre-existing uterine leiomyomata may increase in size during estrogen use. Pathologists should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops, discontinue use while cause is investigated. Administer with caution in patients with impaired liver function, metabolic bone diseases associated with hypercalcemia, or renal insufficiency and in young patients with incomplete bone growth. The following endocrine and liver function tests may be affected by larger estrogen doses: increased sulfobromophthalein retention; increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability; increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay; free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered; impaired glucose tolerance; decreased pregnanediol excretion; reduced response to metyrapone test; reduced serum folate concentration; increased serum triglyceride and phospholipid concentration. *Information for the Patient*—See text of Patient Package Insert. *Pregnancy Category X*—See Contraindications and Boxed Warning. *Nursing Mothers*—Administer to nursing mothers only when clearly necessary.

The 2-mg tablets contain FD&C Yellow No. 5 (tartrazine), which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals, frequently those who also have aspirin hypersensitivity.

Adverse Reactions: (See Warnings regarding induction of neoplasia, adverse effects on the fetus, increased incidence of gall bladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.) The following additional adverse reactions have been reported: *Genitourinary system*—Breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea; premenstrual-like syndrome; amenorrhea during and after treatment; increase in size of uterine fibromyomata; vaginal candidiasis; change in cervical eversion and in degree of cervical secretion; cystitis-like syndrome. *Breast*—Tenderness, enlargement, secretion. *Gastrointestinal*—Nausea, vomiting; abdominal cramps, bloating; cholestatic jaundice. *Skin*—Chloasma or melasma which may persist when drug is discontinued; erythema multiforme, erythema nodosum, hemorrhagic eruption, loss of scalp hair, hirsutism. *Eyes*—Steepening of corneal curvature; intolerance to contact lenses. *CNS*—Headache, migraine, dizziness; mental depression; chorea. *Miscellaneous*—Increase or decrease in weight, reduced carbohydrate tolerance, aggravation of porphyria, edema, changes in libido.

How Supplied: Tablets, containing 1 mg (lavender) and 2 mg (turquoise) micronized estradiol, in bottles of 100 and compacts of 25; Vaginal Cream, each gram containing 0.1 mg 17β-estradiol in a nonliquefying base containing purified water, propylene glycol, stearyl alcohol, white ceresin wax, glyceryl monostearate, hydroxypropyl methylcellulose, sodium lauryl sulfate, methylparaben, disodium edetate, and *t*-butylhydroquinone, in 1½-oz (42.5-g) tubes with calibrated plastic applicator for delivery of 1,2,3, or 4 g and in 1½-oz (42.5-g) refill tubes.

For Prescribing Information, please see physician package insert.

Mead Johnson LABORATORIES

MEAD JOHNSON & COMPANY • EVANSVILLE, INDIANA 47721 USA

MJL 5-4169

C-V14-12-85

OBSTETRICS-GYNECOLOGY

POSITION AVAILABLE IN THE OB-GYN DEPARTMENT OF A 100+ PHYSICIAN MULTISPECIALTY GROUP WITH A WELL ESTABLISHED SATELLITE SYSTEM. 300,000 PATIENT POPULATION BASE. PRACTICE STATE OF THE ART MEDICINE IN A RELATIVELY RURAL AREA WITH NEARBY NATIONAL RECREATION AREA. LARGE CITIES READILY ACCESSIBLE BY PARKWAY. WELL EQUIPPED 400-BED HOSPITAL WITH NEONATAL NURSERY AND FULL RANGE OF ANCILLARY SERVICES. ALL MEDICAL AND SURGICAL SUBSPECIALTIES REPRESENTED ON STAFF. GUARANTEED MINIMUM OF $100,000 ANNUAL SALARY BASE WITH EARLY PARTICIPATION IN THE CLINIC'S PRIMARILY INCENTIVE BASED INCOME DISTRIBUTION SYSTEM. PLEASE SEND CURRICULUM VITAE TO:

Assistant Medical Director
Trover Clinic
Clinic Drive
Madisonville, Kentucky 42431
or
Call Collect: 502-825-7470

FACULTY OPENINGS IN OBSTETRICS AND GYNECOLOGY

1. Perinatology
2. Gynecologic Endocrinology
3. General Obstetrics/Gynecology
4. Family Planning
5. Gynecologic Oncology

Rank and salary: negotiable, commensurate with training and experience.

Qualifications: M.D. with eligibility for certification by American Board of Obstetrics and Gynecology and, where applicable, certification (or eligibility for) in subspecialty is desirable. Interest and experience in undergraduate and graduate education, clinical and/or basic research, and clinical practice of obstetrics and gynecology.

For further information, please contact:

William J. Dignam, M.D.
Acting Chairman
Dept. Obstetrics & Gynecology
UCLA School of Medicine
Los Angeles, California 90024

Affirmative Action/Equal Opportunity Employer

Virginia Baptist Hospital

3300 Rivermont Avenue, Lynchburg, VA 24503
Telephone: 804-522-4000

PERINATOLOGIST/OBSTETRICIANS BOARD CERTIFIED OR ELIGIBLE

Virginia Baptist Hospital is seeking to employ an American trained perinatologist to establish a perinatal regional referral center. The perinatologist will administer the high risk referral system for both the region and private physicians.

Virginia Baptist Hospital has been proposed as one of the seven perinatal centers in Virginia. We average 2,500 deliveries annually and provide full neonatology services. The hospital is currently participating in a state grant serving a region comprised of nine counties with a population 450,000. The obstetrical services and the NICU were relocated in a new addition to the hospital in the fall of 1982 providing a complete scope of services from birthing rooms to cesarean section suite. In addition, Virginia Baptist Hospital is committed to providing a full spectrum of birthing options.

Virginia Baptist Hospital is a full-service JCAH accredited 384-bed hospital located at the foot of the Blue Ridge Mountains in Central Virginia convenient to summer and winter resorts and historic sites. Excellent benefit and salary package inclusive of professional liability coverage.

Submit C.V. to Dianne T. Nash, Vice President, Virginia Baptist Hospital, Rivermont Avenue, Lynchburg, Virginia, 24503 or call (804) 522-4521.

Instructor/Assistant Professor

Department of Obstetrics & Gynecology

Applicant must have completed an accredited residency in Obstetrics and Gynecology and a Fellowship in Reproductive Endocrinology or Maternal Fetal Medicine with special interest in Genetics and/or Sonography. Applicant will participate in students, Resident and Fellow training, clinical care of women at Parkland Memorial Hospital, and basic and clinical research. Must be eligible for Texas licensure. Interested persons send curriculum vitae to:

F. Gary Cunningham, M.D.
Professor and Chairman
Department Obstetrics and Gynecology
University of Texas Health Science Center
5323 Harry Hines Boulevard
Dallas, Texas 75235-9032

CHAIRMAN
DEPARTMENT OF OBSTETRICS AND GYNECOLOGY
UNIVERSITY OF ILLINOIS COLLEGE OF MEDICINE AT ROCKFORD

We seek to appoint at a Professorial level, a Board Certified M.D. in Obstetrics and Gynecology, who will provide academic leadership, sound management and innovative programmatic conceptualization to the educational, research and service elements of our Department of Ob/Gyn. A clinical role is desirable, and in certain areas of specialization i.e. gynecological oncology, a regional opportunity exists through the OB/Gyn University Teaching Service, at our affiliate, Swedish-American Hospital. Applications should be received no later than May 1, 1987, with an anticipated starting date in September, 1987. Nominations and applications should be directed to Robert Klint, M.D., MHA, Chairman, Search Committee, University of Illinois College of Medicine at Rockford, 1601 Parkview Ave., Rockford, IL 61107.

The University of Illinois is an Affirmative Action/Equal Opportunity Employer

JMP-56

Maternal-Fetal Medicine

Hennepin County Medical Center and the University of Minnesota seek a faculty member to teach and conduct research in Maternal-Fetal Medicine. Candidates must have six years post M.D., board qualified or certified in Maternal-Fetal Medicine will be considered at the Assistant Professor level. Recruiting deadline is April 30, 1987.

Hennepin County Medical center and the University of Minnesota are equal opportunity employers and specifically invite and encourage applications from women and minorities.

Address inquiries to:

Bruce A. Work, Jr., M.D.
Professor and Head
Obstetrics and Gynecology
University of Minnesota
Box 395 UMHC
420 Delaware Street S.E.
Minneapolis, Minnesota 55455

CHAIRPERSON
DEPARTMENT OF OBSTETRICS AND GYNECOLOGY

The Knoxville Unit of the University of Tennessee College of Medicine is seeking a Chairperson for the Department of Obstetrics and Gynecology for the University of Tennessee Medical Center at Knoxville. This medical center is located in East Tennessee and is a 602 bed teaching hospital with research facilities. The position requires leadership ability and a record of commitment to patient care, education, and research. The applicant must be board certified in Obstetrics and Gynecology and possess the academic qualifications for Associate Professor or Professor.

Please submit your curriculum vitae to Edward Buonocore, M.D., Chairman, Ad Hoc OB/GYN Advisory Committee, University of Tennessee Medical Center at Knoxville, 1924 Alcoa Highway, Knoxville, TN, 37920.

An Affirmative Action/Title IX/Section 504 Employer.

CHAIRPERSON
DEPARTMENT OF OBSTETRICS/GYNECOLOGY

The University of Nebraska College of Medicine is searching for a Chairperson of Obstetrics and Gynecology. Applications are sought from board certified individuals with established credentials in research and demonstrated leadership in education. Interested applicants should submit a curriculum vitae by March 15, 1987 to: Chairman, Obstetrics and Gynecology Chairman Advisory Committee, Office of the Dean, University of Nebraska College of Medicine, 42nd Street and Dewey Avenue, Omaha, Nebraska 68105. The University of Nebraska is an Affirmative Action/Equal Opportunity Employer.

OBSTETRICIAN/ GYNECOLOGIST

The Smith-Glynn-Callaway Clinic, Inc., located in Springfield, Missouri, is seeking a physician university trained, board eligible or certified in obstetrics/gynecology to join its 66-physician, multispecialty group practice serving a regional population area of 1,000,000+. Competitive salary with bonus/production incentive. Fringe benefits package includes life, health, pension plan, vacation, paid meeting expenses. Four hospitals with combined bed capacity of 1,766. Excellent secondary schools, four universities. Please call or write to Administrator, 3231 South National, Springfield, MO. 65807 or call collect 417-883-7422.

GYNECOLOGIC ONCOLOGIST

The Allentown Hospital, with the 5th largest obstetrical service in Pennsylvania, is actively seeking a board certified or board eligible gynecologic oncologist for private practice and to provide leadership and medical education in gynecologic oncology.

The Hospital, a component of the HealthEast System, is located in the very desirable Lehigh Valley, in close proximity to all major East Coast cities. It sponsors a fully accredited OB/GYN residency program and has a major medical school affiliation. The Hospital co-sponsors a Comprehensive Community Cancer Center and offers a special interest gynecology group from multi-disciplinary specialties. There are no Board certified or Board eligible gynecologic oncologists in the Lehigh Valley, and the position provides a superb opportunity to establish a subspecialty practice and be integratly involved in a major regional teaching program.

Submit C.V. in complete confidence to:

Sze-ya Yeh, M.D.
Chairman, Department of Obstetrics/Gynecology
The Allentown Hospital
17th & Chew Streets
Allentown, Pennsylvania 18102
(215) 778-2412

MATERNAL-FETAL MEDICINE (Board eligible or Certified)

600 Bed University-affiliated Level III Hospital with 3,000 deliveries/year, 24-hour in-house anesthesiology and neonatology/large NICU, active maternal transfer service, perinatal outreach programs, and fully-approved OB/GYN residency program has immediate opening for a full-time position in perinatology. Duties to include residency teaching, maternal transfer care, and private practice incentive. Competitive guaranteed salary plus incentive. Excellent public school system, housing, and Lake St. Clair in very close proximity.

CALL COLLECT: 313-343-3495
SAINT JOHN HOSPITAL
DEPT. OB/GYN
DETROIT, MI 48236

OB-GYN

OBSTETRICIAN - GYNECOLOGIST wanted to join a progressive and prosperous three-man practice. Outstanding opportunity for a well-trained, Board-eligible, OB-GYN physician. This practice is string in High-risk Obstetrics, Infertility, Counseling, etc. Working out of a Level III hospital.

Send C.V. to:

Drs. Trabin, Manko, & Litt
1401 Forum Way #200
West Palm Beach, FL 33401
Phone: 305 689-8780

In intra-abdominal infection...

Consider

Fact No. 1

Bacteroides fragilis is the major anaerobic pathogen[1]

"The most common anaerobic organism is B fragilis, isolated in 80 to 95 percent of patients with colorectal perforation."[2]

Fact No. 2

Against *B fragilis*, CEFOTAN has equal in vitro activity to cefoxitin*[3-10]

Gorbach, Quintiliani, Neu, and others have studied a total of more than 900 isolates of *B fragilis* and determined that CEFOTAN and cefoxitin have equal in vitro activity.*[3-10]

Fact No. 3

Intra-abdominal infection is polymicrobial

Tally and Gorbach report that *B fragilis* is the major anaerobic pathogen, while *Escherichia coli* is the primary aerobic isolate

Fact No. 4

CEFOTAN has superior gram-negative activity compared with cefoxitin[12]

"...spectrum of activity [of CEFOTAN] is distinguished by having the same anti-anaerobe[†] effect as cefoxitin coupled with enhanced activity against Esch. coli and other Enterobacteriaceae."[13]

—C.J.L. Strachan–

*In vitro activity does not necessarily correlate with in vivo effectiveness.
†Most strains of *B distasonis*, *B ovatus*, and *B thetaiotaomicron* are resistant to CEFOTAN.

© 1986 ICI Americas Inc

The Facts

Fact No. 5 CEFOTAN is cost-effective

Twice-daily CEFOTAN can save 45% in treatment costs and single-dose prophylaxis with CEFOTAN saves 63% when compared with a three-dose regimen of cefoxitin.‡

Conclusion The facts now favor CEFOTAN

CEFOTAN is an ideal agent in the treatment of intra-abdominal infections due to susceptible pathogens including *Bacteroides* species such as *B fragilis*, but not *B distasonis*, *B ovatus*, or *B thetaiotaomicron*.

As active as cefoxitin against *Bacteroides fragilis*—the most common anaerobe in intra-abdominal sepsis—CEFOTAN provides superior gram-negative coverage and is particularly active against *E coli* and other common gram-negative pathogens.

In intra-abdominal infection due to indicated organisms Switch to

CEFOTAN® IM/IV
(cefotetan disodium)

The cost-effective replacement for cefoxitin

Please see the following page for brief summary of prescribing information.

‡Based on overall costs of antibiotic preparation and delivery[14] for comparable regimens: Treatment—CEFOTAN, 2 g × 2; cefoxitin,[15] 2 g × 4. Prophylaxis—CEFOTAN 2 g × 1; cefoxitin,[15] 2 g × 3.

CEFOTAN® IM/IV
(cefotetan disodium)

For Intravenous or Intramuscular Use

BRIEF SUMMARY

INDICATIONS AND USAGE
TREATMENT: CEFOTAN® (cefotetan disodium) is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:

Urinary tract infections caused by *E coli*, *Klebsiella* species (including *K pneumoniae*), *Proteus mirabilis* and *Proteus* sp (which may include the organisms now called *Proteus vulgaris*, *Providencia rettgeri*, and *Morganella morganii*).

Lower respiratory tract infections caused by *Streptococcus pneumoniae* (formerly *D pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K pneumoniae*), and *E coli*.

Skin and skin structure infections caused by *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis*,* *Streptococcus pyogenes* and *Streptococcus* species (excluding enterococci), and *E coli*.

Gynecologic infections caused by *Staphylococcus aureus*† (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis*,* *Streptococcus* species (excluding enterococci), *E coli*, *Proteus mirabilis*, *Neisseria gonorrhoeae*, *Bacteroides* species (excluding *B distasonis*, *B ovatus*, *B thetaiotaomicron*), *Fusobacterium* species,† and gram-positive anaerobic cocci (including *Peptococcus* and *Peptostreptococcus* species†).

Intra-abdominal infections caused by *E coli*, *Klebsiella* species (including *K pneumoniae*†), *Streptococcus* species (excluding enterococci), and *Bacteroides* species (excluding *B distasonis*, *B ovatus*, *B thetaiotaomicron*).

Bone and joint infections caused by *Staphylococcus aureus*.†

***NOTE:** Some strains of *Staphylococcus epidermidis* are resistant to cefotetan.

†Efficacy for this organism in this organ system was studied in fewer than ten infections.

PROPHYLAXIS: The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (eg, cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS
CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS
Before therapy with CEFOTAN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefotetan disodium, cephalosporins, penicillins, or other drugs. This product should be given cautiously to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to CEFOTAN occurs, discontinue the drug. Serious acute hypersensitivity reactions may require epinephrine and other emergency measures.

Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with antibiotic use.

Treatment with broad-spectrum antibiotics may alter normal flora of the colon and may permit overgrowth of clostridia. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin in vitro.

Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte, and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance, or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C difficile*. Other causes should also be considered.

PRECAUTIONS
GENERAL: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity. Those at risk include patients with renal or hepatic impairment, or poor nutritional state. Prothrombin times should be monitored in patients at risk and exogenous Vitamin K administered as indicated.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

INFORMATION FOR PATIENTS: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.

DRUG INTERACTIONS: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycoside antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

DRUG/LABORATORY TEST INTERACTIONS: A false positive reaction for glucose in urine may occur with Benedict's or Fehling's solution.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels of Jaffe reaction and produce false increases in the levels of creatinine reported.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approx 8-16 times the usual adult human dose) on days 6-35 of life (thought to be developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approx 2-4 times the usual human dose), only 1 of 10 treated animals was affected, and the degree of degeneration was mild. Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.

USAGE IN PREGNANCY: Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

USAGE IN NURSING MOTHERS: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.

PEDIATRIC USE: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS
In clinical studies the following adverse effects were considered related to CEFOTAN therapy.

Gastrointestinal symptoms occurred in 1.5 percent of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700).

Hematologic laboratory abnormalities occurred in 1.4 percent of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300).

Hepatic enzyme elevations occurred in 1.2 percent of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).

Hypersensitivity reactions were reported in 1.2 percent of patients and included rash (1 in 150) and itching (1 in 700).

Local effects were reported in less than one percent of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500).

DOSAGE AND ADMINISTRATION
TREATMENT: The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6* grams	3 g every 12 hours IV

*Maximum daily dosage should not exceed 6 grams.

PROPHYLAXIS: To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.

IMPAIRED RENAL FUNCTION: When renal function is impaired, a reduced dosage schedule must be employed. The following dosage guidelines may be used.

DOSAGE GUIDELINES FOR PATIENTS WITH IMPAIRED RENAL FUNCTION

Creatinine Clearance mL/min	Dose	Frequency
>30	Usual Recommended Dose*	Every 12 hours
10-30	Usual Recommended Dose*	Every 24 hours
<10	Usual Recommended Dose*	Every 48 hours

*Dose determined by the type and severity of infection, and susceptibility of the causative organism.

Cefotetan is dialyzable and it is recommended that for patients undergoing intermittent hemodialysis, one-quarter of the usual recommended dose be given every 24 hours on days between dialysis and one-half the usual recommended dose on the day of dialysis.

HOW SUPPLIED
CEFOTAN is a dry, white to pale yellow powder supplied in vials containing cefotetan disodium equivalent to 1 g and 2 g cefotetan activity for intravenous and intramuscular administration. The 1 g dose is available in 10 mL and 100 mL vials, and the 2 g dose is available in 20 mL and 100 mL vials. The vials should not be stored at temperatures above 22°C and should be protected from light.
1 g in 10 mL vial (NDC 0038-0376-10)
2 g in 20 mL vial (NDC 0038-0377-20)
1 g in 100 mL vial (NDC 0038-0376-11)
2 g in 100 mL vial (NDC 0038-0377-21)

Manufactured for



STUART PHARMACEUTICALS
Division of ICI Americas Inc
Wilmington, Delaware 19897

Rev E 3/86 63967-02

References: 1. Gorbach SL: Bacteroides species, in Mandel GL, Douglas RG Jr, Bennett JE (eds): *Principles and Practice of Infectious Diseases*, ed 2. New York, John Wiley & Sons, 1985, p 1370. 2. Simmons RL, Howard RJ, Henriksen AI (eds): *Surgical Infectious Diseases*. New York, Appleton-Century-Crofts, 1982, p 922. 3. Souza Dias MB, Jacobus NV, Tally FP, Gorbach SL: Activity of cefotetan against anaerobic bacteria. *Diagn Microbiol Infect Dis* 1936;4:359-363. 4. Data on file, Stuart Pharmaceuticals, Wilmington, Delaware. 5. Neu HC, Chin NX: The activity and beta-lactamase stability of cefotetan compared to other beta-lactam antibiotics. *Chemioterapia* 1985;4:271-277. 6. Hennessey TD: Cefotetan: A compilation of susceptibility data of clinical isolates, in Lode H, Periti P, Strachan CJL (eds): *Cefotetan: A Long-Acting Antibiotic for Polymicrobial Infections*. Proceedings of a Symposium Held in Association With the 4th Mediterranean Congress of Chemotherapy, Rhodes, Greece, 1984. New York, Churchill Livingstone, 1985, pp 14-22. 7. Aldridge KE, Sanders CV, Janney A, et al: Comparison of the activities of penicillin G and new β-lactam antibiotics against clinical isolates of *Bacteroides* species. *Antimicrob Agents Chemother* 1934;26:410-413. 8. Watt B, Brown FV: The comparative *in-vitro* activity of cefotetan against anaerobic bacteria. *J Antimicrob Chemother* 1985;15:671-677. 9. Ruckdeschel G: Activity *in vitro* of cefotetan against non-sporing anaerobes: A comparative study. *J Antimicrob Chemother* 1983;11(suppl A):117-124. 10. Werner H: Inhibitory activity of cefotetan and other β-lactams against anaerobes. *J Antimicrob Chemother* 1983;11(suppl A):107-115. 11. Tally FP, Gorbach SL: Therapy of mixed anaerobic-aerobic infections: Lessons from studies of intra-abdominal sepsis. *Am J Med* 1985;78(suppl 6A):145-153. 12. Ayers LW, Jones RN, Barry AL, et al: Cefotetan, a new cephamycin: Comparison of in vitro antimicrobial activity with other cephems, β-lactamase stability, and preliminary recommendations for disk diffusion testing. *Antimicrob Agents Chemother* 1982;22:859-877. 13. Strachan CJL: Postscript, in Lode H, Periti P, Strachan CJL (eds): *Cefotetan: A Long-Acting Antibiotic for Polymicrobial Infections*. Proceedings of a Symposium Held in Association With the 4th Mediterranean Congress of Chemotherapy, Rhodes, Greece, 1984. New York, Churchill Livingstone, 1985, p 289. 14. McCue JD: *Cost-Conscious Use of the Cephalosporins*. New York, Raven Health Care Communications, 1985, p 37. 15. Direct price from *Drug Topics Red Book*, ed 90. Oradell, NJ, Medical Economics Co, 1986, p 378.

Maternal age and primary cesarean section rates: A multivariate analysis

Michelle Martel, M.D., Sholom Wacholder, Ph.D., Abby Lippman, Ph.D., John Brohan, B.Sc., and Emily Hamilton, M.D.C.M.

Montreal, Quebec, Canada

To study the effect of maternal age on primary cesarean section rates, 3458 consecutive deliveries were analyzed. The cesarean section rates in primiparous women less than 25, 25 to 34, and over 34 years of age were 13.1%, 18.5%, and 28.2%, respectively. A similarly dramatic rise with advancing maternal age was seen in multiparous women with rates of 3.4%, 4.7%, and 10.1%, respectively, in the three age groups. The strong association between cesarean section and maternal age persisted after multivariate adjustment for induction of labor, epidural anesthesia, meconium-stained amniotic fluid, and fetal distress, and thus these factors do not explain the relationship. (Am J Obstet Gynecol 1987;156:305-8.)

Key words: Cesarean section, primary; maternal age

Cesarean section rates have been increasing steadily in Canada, from 6.0% in 1970 to 17.1% in 1983.[1] Little consensus exists on the reasons for this increase. One suggestion has been that the increasing age of the obstetric population contributes to this trend.[2,3] Wadhera and Nair[1] reported a steady rise in the total cesarean section rate with maternal age. Nevertheless, because age-related primary rates are not available and because repeat cesarean sections, which would be more frequent among older women, are not excluded from most statistical summaries, the precise relationship between maternal age and cesarean section rates is unknown and explanations of it can only be speculative. This study was designed to analyze directly the effects of maternal age on primary cesarean section rates, taking into consideration other factors that might vary with maternal age.

Material and methods

All deliveries at the Sir Mortimer B. Davis Jewish General Hospital in Montreal, occurring from April 1984 to August 1985, were reviewed. After exclusion of those that were complicated by multiple gestation, stillbirth, placenta previa, or breech presentation and those in which cesarean section was performed because of a previous cesarean section, 3458 deliveries remained for analysis. These were grouped into three maternal age categories and according to whether the woman was primiparous or multiparous.

For each of the deliveries, information on the presence or absence of meconium, induction of labor, epidural anesthesia, fetal distress, maternal diabetes, hypertension, 1-minute Apgar scores, and birth weight was taken from the computerized delivery record completed at the time of delivery.

Various clinical methods are used to assess fetal health. Electronic monitoring of fetal heart rate was done in all patients in whom the diagnosis of fetal distress was made. Fetal scalp blood or cord blood samples were only rarely available. The antepartum diagnosis of fetal distress was made if there were persistent decelerations that may have been associated with changes in variability of the baseline fetal heart rate. Decreased variability alone was not considered fetal distress. The category of hypertension included all cases of hypertension requiring treatment and those in which the blood pressure was persistently >140/90 mm Hg in labor. The 1- and 5-minute Apgar scores were used to assess infants in whom the diagnosis of fetal distress was made. Because all cesarean sections for fetal distress were attended by neonatal staff, it was considered that the 5-minute Apgar scores would be influenced by resuscitation efforts and would mask the effect of fetal distress.

Analyses were performed separately for primiparous and multiparous women. Crude odds ratios were used as a preliminary measure of the association between maternal age and cesarean section. Because other factors related to maternal age and cesarean section could distort the association between these two variables, we carried out a multivariate logistic regression analysis wherein the dependent variable was presence or ab-

From the Department of Obstetrics and Gynecology, Sir Mortimer B. Davis Jewish General Hospital, and the Department of Epidemiology and Biostatistics, Faculty of Medicine, and the School of Computer Science, McGill University.

Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.

Reprint requests: Dr. E. Hamilton, Sir Mortimer B. Davis Jewish General Hospital, 3755 Cote Ste. Catherine Road, Montreal, Quebec, Canada H3T 1E2.

Table I. Primary cesarean section rates in the various age and parity groups

	Maternal age					
	≤24 yr		*25 to 34 yr*		*≥35 yr*	
	Total No.	*Cesarean section (%)*	*Total No.*	*Cesarean section (%)*	*Total No.*	*Cesarean section (%)*
Primiparous women	602	13.1	1017	18.5	99	28.2
Multiparous women	261	3.4	1201	4.7	278	10.1

Table II. Frequency of obstetric factors related to cesarean section

	Maternal age					
	≤24 yr		*25 to 34 yr*		*≥35 yr*	
Obstetric factor	*Primiparous women (N = 602)*	*Multiparous women (N = 261)*	*Primiparous women (N = 1017)*	*Multiparous women (N = 1201)*	*Primiparous women (N = 99)*	*Multiparous women (N = 278)*
Induction of labor	13.9	6.1	15.2	11.8	13.1	11.8
Epidural anesthesia	54.2	21.4	55.6	26.3	51.5	26.2
Meconium	11.9	11.1	12.7	9.0	14.1	13.3
Fetal distress	3.8	1.1	5.0	1.7	8.1	2.2
Diabetes	1.8	0.8	3.0	2.7	6.1	4.3
Hypertension	5.8	1.1	5.6	2.3	7.1	1.4
Macrosomia (>4000 gm)	7.4	8.8	7.3	9.5	5.1	13.7

Frequency expressed as percent.

Table IIIA. Frequency of the three major indications for cesarean section in primiparous women

	Maternal age		
Indication	*≤24 yr*	*25 to 34 yr*	*≥35 yr*
Dystocia	7.1	11.3	11.1
Fetal distress	3.3	4.6	8.1
Preeclampsia	1.3	1.1	3.1

Frequency expressed as percent.

Table IIIB. Frequency of the three major indications for cesarean section in multiparous women

	Maternal age		
Indication	*≤24 yr*	*25 to 34 yr*	*≥35 yr*
Dystocia	0.8	1.6	5.0
Fetal distress	1.1	1.4	1.8
Abruptio placentae	—	0.2	1.1

Frequency expressed as percent.

sence of cesarean section and the independent variables were meconium staining of the amniotic fluid, induction of labor, epidural anesthesia, and fetal distress.[4] Adjusted odds ratio estimates were obtained from the regression coefficients of the model. These adjusted odds ratio estimates are free from the confounding effects of the other variables in the model. Diabetes and macrosomia were not themselves indications for cesarean section and therefore were not considered as covariates.

To investigate whether different diagnostic standards were applied to older women, we looked at the frequencies of low 1-minute Apgar scores, a marker of fetal distress, in the different age groups. If the diagnosis of fetal distress was applied similarly to women of different ages, the frequency of low 1-minute Apgar scores should be the same in all age groups among women undergoing cesarean section for fetal distress.

Results

Women aged 35 years and older constituted 10.9% of the total population, representing 5.8% of the primiparous women and 16.0% of the multiparous women. The overall cesarean section rate was 11.2% and the rates for each age-parity group are presented in Table I. There was a striking increase in rates in the primiparous women ($\chi^2 = 15.8$, $p < 0.0005$). Cesarean section rates were lower in the multiparous women, but there was still evidence of a significant increase with age ($\chi^2 = 12.2$, $p < 0.0005$).

Table IVA. Crude and adjusted* odds ratio estimates of the association between maternal age and cesarean section in primiparous women

Age (yr)	Crude		Adjusted*	
	Estimate	SE	Estimate	SE
25-34†	1.50	0.22	2.00	0.32
>35†	2.61	0.66	3.56	0.97

*Adjusted for the presence or absence of induction of labor, epidural anesthesia, meconium, and fetal distress.
†Compared with women under 25 years of age.

Table IVB. Crude and adjusted* odds ratio estimates of the association between maternal age and cesarean section in multiparous women

Age (yr)	Crude		Adjusted*	
	Estimate	SE	Estimate	SE
25-34†	1.32	0.48	1.27	0.55
>35†	3.14	1.23	3.49	1.58

*Adjusted for the presence or absence of induction of labor, epidural anesthesia, meconium, and fetal distress.
†Compared with women under 25 years of age.

Table V. Frequency of 1-minute Apgar scores of ≤5 in infants of women having cesarean section for fetal distress

	Maternal age					
	≤24 yr		25 to 34 yr		≥35 yr	
	No.	%	No.	%	No.	%
Primiparous women	5/20	20.0	11/47	23.4	2/8	25.0
Multiparous women	1/3	33.3	4/17	23.5	1/5	20.0

A number of obstetric factors that may be associated directly or indirectly with a higher probability of cesarean section and their distribution according to maternal age are shown in Table II. As can be seen, there were no major differences in the frequencies of induction of labor, epidural anesthesia, or meconium in the various age or parity groups. Although fetal distress appeared to occur more frequently in the oldest age group, its relative rarity suggests that it cannot account for the increasing cesarean section rates.

Rates of dystocia among all of the age groups are presented in Tables IIIA and IIIB. It should be noted that dystocia is poorly defined clinically and the term is generally applied only when a cesarean section is performed. As a result it is not a useful covariate.

Dystocia, fetal distress, preeclampsia, and abruptio placentae were the major indications for cesarean section in this selected study population and their frequencies in the various age groups are shown in Tables IIIA and IIIB. These indications account for 84.5% and 71.0%, respectively, of all the cesarean sections in the primiparous and multiparous women. None of the other indications for cesarean section occurred in more than 2% of the women in this selected population.

Crude and adjusted odds ratios together with their standard errors are found in Tables IVA and IVB. For example, in Table IVA the crude estimate of the odds ratio in women aged 25 to 34 years shows them to be 1.5 times more likely to have a cesarean section than are primiparous women under 25 years of age. When the analysis is done by removing the effects of possibly confounding covariates, these same women are found to be 2.0 times more likely to have a cesarean section than are women under 25 years of age. The age effect is highly significant ($p < 0.0005$) for all of the analyses. The odds ratios are not substantially affected by the inclusion of the adjusting factors, suggesting that these factors alone or in combination do not explain the association between age and cesarean section.

The frequencies of low 1-minute Apgar scores among infants of women having a cesarean section for fetal distress were similar in the three age groups of primiparous and multiparous women (Table V). From this distribution, it would appear that fetal distress is being diagnosed similarly in the older and younger women even though it is more common among the older women.

Comment

Analysis confirms the association between maternal age and cesarean section rates, with dramatic increases seen among both primiparous and multiparous women. Results of multivariate analysis indicate that the increased rates are not an artifact resulting from the effects of the chosen covariates inasmuch as the adjusted rates do not vary substantially from the crude rates. In other words, even after adjustment for the covariates, the strong association between maternal age and cesarean section persists.

This analysis was restricted to variables available rou-

tinely from the delivery record. It is possible that unmeasured factors could explain the age and cesarean section association. These factors could include socioeconomic status, parental anxiety, or previous infertility, for example. It is unlikely, however, that they would include medical factors, since these would be subsumed in the chosen covariates.

It may also be that the increased rate in older women results from physician beliefs that this group is at increased risk for labor complications and that cesarean section can circumvent these problems. This is in agreement with the observations of Kirz et al.[5] More needs to be learned about how physicians make management decisions and whether these decisions are the most appropriate for older women.

REFERENCES

1. Wadhera S, Nair C. Trends in cesarean section deliveries, Canada, 1968-1977. Can J Public Health 1982;73:47.
2. Bottoms SF, Rosen MG, Sokol RJ. The increase in the cesarean birth rate. N Engl J Med 1980;302:559.
3. Anderson GM, Lomas J. Determinants of the increasing cesarean birth rate. N Engl J Med 1984;311:887.
4. Anderson S, Augier A, Havek WW, et al. Statistical methods for comparative studies. New York: John Wiley, 1980.
5. Kirz DS, Dorchester W, Freeman RK. Advanced maternal age: the mature gravida. AM J OBSTET GYNECOL 1985; 152:7.

Prenatal diagnosis and management of congenital defects of the anterior abdominal wall

M. Sermer, M.D., R. J. Benzie, M.D., L. Pitson, M.D., M. Carr, M.D., and M. Skidmore, M.D.

Toronto, Ontario, Canada

Between the years 1980 and 1985, 25 cases of anterior abdominal wall defects were identified within the University of Toronto Perinatal Complex. There were 17 cases of omphalocele and eight cases of gastroschisis. Associated anomalies were found in 71% of infants with omphalocele and 50% with gastroschisis. They were the major cause of neonatal death. Prematurity was the second most common cause of death. The neonatal death rate was 59% in omphalocele and 38% in gastroschisis; the prematurity rates were 53% and 50%, respectively. In omphalocele, there was a 47% cesarean section rate, with a 50% neonatal death rate. Vaginal delivery was associated with a 67% death rate. In gastroschisis, there was a 50% cesarean section rate, with a 50% neonatal death rate. Vaginal delivery was associated with a 25% death rate. There is no evidence that cesarean section offers improved neonatal survival. (AM J OBSTET GYNECOL 1987;156:308-12.)

Key words: Omphalocele, gastroschisis, prenatal diagnosis, ultrasound

The incidence of omphalocele is in the order of one in 4000 to one in 5000 live births, while that of gastroschisis is about one in 10,000 to one in 12,000 live births.[1, 2] Even though the incidence is relatively low, increased neonatal survival in the past decade has resulted in greater attention being paid to these congenital abnormalities.

These increased survival rates are secondary to advances within neonatal intensive care units, improvement in intravenous nutrition, better infection control, and development of new surgical techniques. For this reason, greater attention must be paid to the antenatal diagnosis, counseling, and management of these conditions.

Omphalocele is a defect of the umbilical ring and medial segments of the two embryonic lateral abdmonal wall folds. A sac is present into which intra-abdominal, and occasionally intrathoracic, contents may herniate. The inner layer of the sac consists of peritoneum and the outer layer consists of amnion. The umbilical cord insertion arises from the sac.

Gastroschisis is characterized by intestinal herniation through a defect in the anterior abdominal wall. This defect is usually to the right of the umbilicus. The um-

From the University of Toronto Perinatal Complex, Women's College Hospital, Toronto General Hospital.
Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.
Reprint requests: Dr. M. Sermer, Department of Obstetrics and Gynaecology, Toronto General Hospital, 101 College St., Toronto, Ontario, Canada.

Table I. Omphalocele: Associated anomalies and neonatal outcomes

Case No.	*Chromosome analysis after amniocentesis*	*Associated anomalies*	*Outcome*
1	Trisomy 18	Patent ductus arteriosus, patent foramen ovale, renal dysplasia, craniofacial-skeletal anomalies	Neonatal death
2	Trisomy 18	Tetralogy of Fallot, scoliosis	Stillbirth
3	Trisomy 13	Atrial septal defect, ventricular septal defect, hypoplastic genitalia, craniofacial anomalies	Neonatal death
4	46,XX (17p-)	Cardiomegaly, neural tube defect	Died at 10 mo of congestive heart failure
5	Normal	Pulmonary artery stenosis, coarctation of aorta, undescended testicles, pulmonary hypoplasia	Neonatal death (respiratory failure)
6	Not done	Ectopia cardis, bifid ureter, hypoplastic lung, uterine anomalies, skeletal anomalies	Neonatal death
7	Not done	None	Neonatal death (respiratory distress syndrome, prematurity)
8	Not done	None	Neonatal death (respiratory distress syndrome, prematurity)
9	Not done	Meckel's diverticulum	Died at 31 days of bowel obstruction
10	Not done	None	Stillbirth (severe abruptio placentae)
11	Normal	Tetralogy of Fallot, pulmonary artery stenosis	Survived (respiratory distress syndrome)
12	Normal	Rectovesical fistula, short gut syndrome, imperforate anus, ambiguous genitalia	Survived (cardiac arrest)
13	Normal	Anal stenosis, cloacal exstrophy, ambiguous genitalia	Survived
14	Not done	Ileal atresia, blepharophimosis, syndactyly	Survived
15	Normal	Hypospadias	Survived
16	Normal	None	Survived (respiratory distress syndrome)
17	Not done	None	Survived

bilicus is not part of the defect. There is no sac covering the intestines. The intestines are thickened, edematous, and covered by an inflammatory exudate. These changes are thought to be secondary to the irritating effect of the amniotic fluid (pH 7.0).[3]

Ultrasound evaluation of omphalocele should include the assessment of the size of the sac and the entry of the umbilical vessels into the sac. Accurate assessment of the herniated organs should be made. In addition to intestines, organs that herniate may include the stomach, the liver, the spleen, and the heart. The umbilical cord enters the abdominal wall normally in gastroschisis. There is no sac present and the thickened intestines flow freely within the amniotic cavity. The herniated bowel will result in a cauliflower-like appearance.[4] Occasionally stomach and internal female genitalia may herniate as well.

The purpose of this article is to summarize our experience, review current literature, and then offer a reasonable outline for the prenatal diagnosis and management of these patients.

Method

A 5-year experience (October 1980 to November 1985) of four tertiary referral centers within the University of Toronto Perinatal Complex has been reviewed. Only the cases of abdominal wall defects in which it was elected to continue with the pregnancy were evaluated. Twenty-five cases were identified. Eight of these were transfered to the University of Toronto Perinatal Complex from outside centers.

Prematurity was defined as <37 completed weeks' gestation. Small for gestational age was defined as actual birth weight below the ninetieth percentile for the given gestational age.[5]

Results

A total of 25 cases of abdominal wall defects were identified (17 with omphalocele and eight with gastroschisis). None of the patients had prenatal serum levels of α-fetoprotein estimated. Ultrasound was performed in 65% (11 of 17) of cases of omphalocele and 75% (six of eight) of cases of gastroschisis. Of those that had prenatal ultrasound evaluations, the diagnosis was made in 64% (seven of 11) of the cases of omphalocele and 33% (two of six) of the cases of gastroschisis.

Associated anomalies were documented in 71% of infants with omphalocele and in 50% of infants with gastroschisis. The most common anomalies associated with omphalocele were: genitourinary, 47% (eight of 17); cardiac, 41% (seven of 17); chromosomal, 40% (four, only 10 tested); musculoskeletal, 29% (five of 17); gastrointestinal, 29% (five of 17); and central nervous

Table II. Gastroschisis: Associated anomalies and neonatal outcomes

Case No.	*Chromosome analysis after amniocentesis*	*Associated anomalies*	*Outcome*
1	Not done	None	Neonatal death (fetal distress, birth asphyxia)
2	Normal	Cleft palate	Neonatal death (respiratory distress syndrome, prematurity)
3	Not done	Large segments of small bowel atresia	Neonatal death (necrotic bowel)
4	Not done	Jejunal stenosis	Survived
5	Not done	Diaphragmatic hernia	Survived
6	Not done	None	Survived
7	Not done	None	Survived
8	Not done	None	Survived

Table III. Prematurity and rate of small for gestational age

	Rate of small for gestational age		*Prematurity rate*	
	%	*n*	%	*n*
Omphalocele	35	6/17	53	9/17
Gastroschisis	29	2/7*	50	4/8

*In one patient no birth weight was available.

Table IV. Omphalocele: Liver herniation and neonatal outcome

	Death rate	
	%	*n*
Liver herniation	88	7/8
No liver herniation	33	3/9

system, 6% (one of 17) (Table I). In contrast, there were only four anomalies in fetuses with gastroschisis. These were diaphragmatic hernia, cleft palate, jejunal stenosis, and small bowel atresia. The latter two are a complication of gastroschisis itself (Table II).

The neonatal death rate was 59% (10 of 17) in cases of omphalocele (six deaths due to associated anomalies, two due to prematurity, one due to obstetric complications, and one due to postoperative complications after repair of omphalocele) (Table I).

In omphalocele there were 12 cases with associated anomalies. Neonatal death occurred in seven, for a 58% death rate. No infant with a chromosomal anomaly survived. Six of seven infants with cardiac abnormalities died (four of the cardiac lesions were associated with chromosomal abnormalities). Two of five infants with gastrointestinal and four of eight infants with genitourinary anomalies died; however, these deaths were secondary to other associated conditions. Musculoskeletal abnormalities were seen in five cases, and four of these infants died. One infant with central nervous system abnormality died. However, these deaths were also secondary to other associated anomalies (Table I).

There were five cases with no associated abnormalities. Two infants died of prematurity and one died of severe abruptio placentae. The neonatal death rate in this group was 60% (three of five) (Table I).

There was a 38% (three of eight) death rate in cases of gastroschisis (one due to bowel atresia, one due to prematurity, and one due to an obstetric complication) (Table II).

In gastroschisis there were four patients with no associated abnormalities. Only one died, secondary to birth asphyxia (Table II).

The prematurity rates (<37 weeks' gestation) for omphalocele and gastroschisis were 53% and 50%, respectively. The infants were small for gestational age in 35% of cases of omphalocele and in 29% of cases of gastroschisis (Table III).

Table IV indicates that liver herniation is associated with an 88% (seven of eight) neonatal death rate in cases of omphalocele. Three patients, in addition to liver herniation, had the spleen, stomach, and heart within the omphalocele sac. In all of these cases neonatal death was recorded. Neonatal death was reported in one case of gastroschisis where the liver herniated.

In omphalocele, there was a 47% cesarean section rate, with a 50% neonatal death rate. Vaginal delivery was associated with a 67% death rate. In gastroschisis, there was a 50% cesarean section rate, with a 50% neonatal death rate. Vaginal delivery was associated with a 25% death rate (Table V).

Comment

This is a descriptive study of 25 cases of abdominal wall defects. None of the results obtained here showed a statistically significant difference, as measured by the 95% confidence interval or χ^2 test. Confidence in the results can be obtained only after prospective controlled trials are performed. Because of the low incidence of this condition such trials would be difficult to obtain and none are currently available. Nevertheless, some trends are becoming apparent from this article. Recommendations are based on these trends and

Table V. Mode of delivery and neonatal death rate

	Caesarean section		*Vaginal delivery*		*Total*	
	%	*n*	%	*n*	%	*n*
Omphalocele	50	4/8	67	6/9	59	10/17
Gastroschisis	50	2/4	25	1/4	38	3/8

on the information available from the current literature.

This study shows that the overall accuracy of identifying abdominal wall defects by ultrasound was only 64% for omphalocele and 34% for gastroschisis. Anomalies were missed in both the second and the third trimesters. This low detection rate is most likely due to the fact that some of these were routine ultrasound examinations performed at primary care centers. The value of detailed ultrasound, as an accurate tool in the diagnosis of abdominal wall defects, has been adequately documented.[3, 4, 6-8] A careful ultrasound examination cannot be overemphasized.

Ultrasound should be used to differentiate between the two conditions, by the well-established criteria.[4] The size of the lesion should be established, as excessively large lesions have been said to result in dystocia and rupture of the sac. More importantly, identification of herniated organs should be documented. In this study liver herniation in omphalocele was associated with an 88% neonatal death rate (Table IV). Spleen and heart herniation was associated with a 100% death rate. This finding is supported by Kirk et al.[10]

Ultrasound should be used to screen for other associated anomalies. In this study, 71% of cases of omphalocele were associated with other anomalies. Other large studies have reported slightly lower rates, between 29% and 66%.[1-3, 10-12] In this review, the only gastroschisis-associated anomaly that could be detected by ultrasound was diaphragmatic hernia.

Antenatal monitoring of the patients with continuing pregnancies should include serial ultrasound evaluations, not only to monitor abdominal wall defects but also to identify small for gestational age infants. The rate of small for gestational age in omphalocele was 35%; in gastroschisis it was 29%. Carpenter et al.[2] found 20% and 77% rates of intrauterine growth retardation in omphalocele and gastroschisis, respectively.

As there is an increased risk of premature labor and delivery, parents should be counseled about the possibility of premature labor so that appropriate monitoring and therapeutic measures can be instituted. The prematurity rate was about 50% in both omphalocele and gastroschisis. An increased prematurity rate was also noted by Grosfeld et al.[3] and Mayer et al.[13]

Omphalocele but not gastroschisis is associated with major chromosomal abnormalities.[2-4, 11-13] These were seen in 40% of patients where amniocentesis was performed. Neonatal death was recorded in all of these infants. Therefore, in cases where omphalocele is suspected, amniocentesis is indicated.

Major cardiac abnormality was seen in 41% of cases of omphalocele, an incidence comparable to that of others.[1-3, 12, 13] Cardiac abnormalities in gastroschisis cases are much less common. The highest rate was reported by Mann et al.,[12] where two of 16 patients had a cardiac anomaly. Others[2, 3, 13, 14] report rates of 0% to 8%. As the mortality rate associated with omphalocele and cardiac defect is 86%, fetal echocardiography should be performed. Further support for such investigation is offered by Crawford et al.[15]

In the past it was thought that cesarean section offered a better survival rate. For that reason several investigators elected to perform cesarean delivery in cases where abdominal wall defects were diagnosed prenatally.[16] Currently several authors[10, 14] have indicated that there is no clear evidence that cesarean section offers improved survival. Rupture of the sac is a relatively rare event and was seen in none of the omphalocele cases reported here. More importantly, neonatal death in all of the cases could be attributed to other complicating factors, mainly associated anomalies and prematurity.

In infants with no associated anomalies good outcome can be anticipated. This is particularly true of the infants with gastroschisis. These infants are still at risk of neonatal death secondary to prematurity, obstetric complications, and postoperative complications. Most common postoperative complications include aspiration pneumonia, sepsis, and small bowel obstruction and infarction.

Infants with abdominal wall defects should preferably be delivered in a tertiary pediatric center with surgical experience to correct these defects.

In conclusion, because of current medical advances, an increasing number of infants afflicted with abdominal wall defects are now surviving. For this reason it is important to identify prenatally those infants where a good prognosis can be anticipated. Where the prognosis is grave, because of the anomalies, a decision may have to be made about terminating the pregnancy.

Fig. 1 shows a suggested plan of management for abdominal wall defects. The maternal serum α-fetoprotein level has been shown to be elevated in abdominal wall defects.[12, 17] Differential diagnosis of elevated α-fetoprotein values, seen in patients who are being

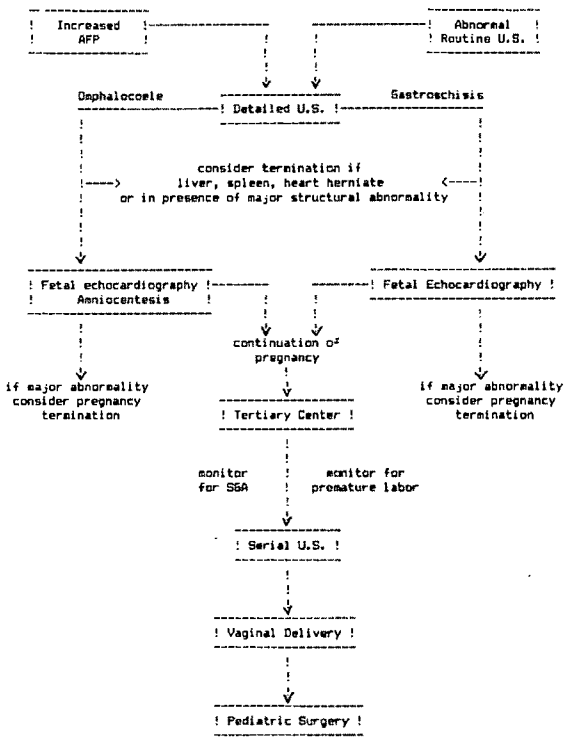


Fig. 1. Plan of management for abdominal wall defects.

screened for neural tube defects, should include abdominal wall defects. Detailed ultrasound should therefore be performed not only to look for neural tube defects but also to assess the integrity of the anterior abdominal wall. Herniated organs and other structural abnormalities should also be assessed. Termination of pregnancy should be considered in the presence of liver, spleen, and heart herniation.

Should routine ultrasound show the presence of abdominal wall defects a similar detailed ultrasound evaluation should be performed. Fetal echocardiography should be performed in both gastroschisis and omphalocele cases. Amniocentesis is indicated only in omphalocele. Should the pregnancy continue careful monitoring for premature labor and for appropriate fetal growth should be carried out. Delivery should be performed in tertiary care centers with appropriate pediatric backup. As there is no evidence that cesarean section offers improved survival, vaginal delivery should generally be performed.

REFERENCES

1. Baird PA, MacDonald EC. An epidemiologic study of congenital malformations of the anterior abdominal wall in more than a million consecutive live births. Am J Hum Genet 1981;33:470-8.
2. Carpenter MW, Curci MR, Dibbins AW, Haddow JE. Perinatal management of ventral wall defects. Obstet Gynecol 1984;64:646-51.
3. Grosfeld JL, Dawes L, Weber TR. Congenital abdominal wall defects: current management and survival. Surg Clin North Am 1981;61:1037.
4. Redford DHA, McNay MB, Whittle MJ. Gastroschisis and exomphalos: precise diagnosis by midpregnancy ultrasound. Br J Obstet Gynaecol 1985;92:54-9.
5. Brenner WE, Edelman DA, Hendricks CH. A standard of fetal growth for the United States of America. AM J OBSTET GYNECOL 1976;126:555.
6. Jauppila P, Kirkinen P. Ultrasonic and clinical aspects in the diagnosis and prognosis of congenital gastrointestinal anomalies. Ultrasound Med Biol 1984;10:465-72.
7. Bovicelli L, Rizzo N, Orsini LF, Pilu G. Prenatal diagnosis and management of fetal gastrointestinal abnormalities. Semin Perinatol 1983;7:109-17.
8. Canty TG, Leopold GR, Wolf DA. Maternal ultrasonography for the antenatal diagnoses of surgically significant neonatal anomalies. Ann Surg 1981;194:353.
9. Hobbins JC, Venus I, Tortora M, Mayden K, Mahoney MJ. Stage II ultrasound examination for the diagnosis of fetal abnormalities with an elevated amniotic fluid alpha-fetoprotein concentration. AM J OBSTET GYNECOL 1982; 142:1026-9.
10. Kirk EP, Wah RM. Obstetric management of the fetus with omphalocele or gastroschisis: a review and report of one hundred twelve cases. AM J OBSTET GYNECOL 1983; 146:512-8.
11. Knight PJ, Buckner D, Vassy LE. Omphalocele: treatment options. Surgery 1981;89:332-6.
12. Mann L, Ferguson-Smith MA, Desai M, Gibson AAM, Raine PAM. Prenatal assessment of anterior abdominal wall defects and their prognosis. Prenat Diagn 1984; 4:427-35.
13. Mayer T, Black R, Matlak ME, Johnson DG. Gastroschisis and omphalocele. Ann Surg 1980;192:783-7.
14. Davidson JM, Johnson TRB Jr, Rigdon DT, Thompson BH. Gastroschisis and omphalocele: prenatal diagnosis and perinatal management. Prenat Diagn 1984;4: 355-63.
15. Crawford DC, Chapman MG, Allan LD. Echocardiography in the investigation of anterior abdominal wall defects in the fetus. Br J Obstet Gynaecol 1985;92:1034-6.
16. Cameron GM, McQuown DS, Modanlou HD, Zemlyn S, Pillsbury SG. Intrauterine diagnosis of an omphalocele by diagnostic ultrasonography. AM J OBSTET GYNECOL 1978; 131:821-2.
17. Norgaard-Pedersen B, Bagger P, Bang J, et al. Maternal serum alphafetoprotein screening for fetal malformations in 28062 pregnancies. Acta Obstet Gynecol Scand 1985; 64:511-4.

Congenital heart disease in pregnancy: Short- and long-term implications

J. Shime, M.D., E. J. M. Mocarski, M.Sc., M.D., D. Hastings, M.D., G. D. Webb, M.D., and P. R. McLaughlin, M.D.

Toronto, Ontario, Canada

One hundred forty-four pregnancies that occurred in 74 patients with congenital heart disease at the Toronto General Hospital between 1975 and 1986 were reviewed. The patients were divided into two groups, those with acyanotic lesions and those with cyanotic lesions. Maternal and fetal outcomes in these two groups were compared. Patients with cyanotic lesions developed significantly more congestive heart failure and deteriorated more often in functional cardiac status than those with acyanotic lesions ($p < 0.005$). Women with obstructive lesions had a higher incidence of pregnancy-induced hypertension than our general population ($p < 0.005$). There was one maternal death in the puerperium in a woman with Eisenmenger's syndrome. Preterm births were more frequent in the cyanotic group ($p < 0.01$) as were small for gestational age infants ($p < 0.005$). In the cyanotic group the mean birth weight was 900 gm less than that in the acyanotic group ($p < 0.005$). The major benefits of corrective cardiac surgery were in the cyanotic group and consisted of fewer spontaneous abortions ($p < 0.01$) and small for gestational age infants ($p < 0.02$) and more term births ($p < 0.01$). Long-term follow-up revealed that 19.2% of women with acyanotic lesions had significant cardiac developments, compared with 90% in the cyanotic group ($p < 0.005$). One of 87 infants (1.1%) had congenital heart disease and two had Marfan's syndrome. (Am J Obstet Gynecol 1987;156:313-22.)

Key words: Congenital heart disease, heart, maternal, fetal

The ratio of rheumatic to congenital heart disease in pregnancy at the Toronto General Hospital has gone from 4:1 in 1962[1] to unity in 1985. This change can be partly attributed to an active adult congenital heart clinic that receives referrals from the adjacent The Hospital For Sick Children. In general, however, the declining incidence of rheumatic heart disease combined with advances in the surgical and medical management of congenital heart disease is responsible and reflects a global trend. Thus maternal heart disease, the leading nonobstetric cause of death in pregnancy, is changing and requires a reappraisal in terms of risks and management.

The following report documents our experience with congenital heart disease during pregnancy at the Toronto General Hospital from January 1975 to August 1986. Since the maternal physical condition will, at least in part, influence the ability to perform the tasks of motherhood, long-term evaluation of several patients has also been obtained. Finally, inasmuch as the offspring of these women are themselves at increased risk for congenital heart disease, all infants underwent examination for cardiac defects.[2]

From the Divisions of Maternal-Fetal Medicine and Cardiology, Toronto General Hospital, and the Departments of Obstetrics and Gynecology and Medicine, the University of Toronto.

Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.

Reprint requests: Dr. J. Shime, Women's College Hospital, 602 Burton Hall, 60 Grosvenor St., Toronto, Ontario, Canada M5S 1B6.

Patients and methods

Seventy-four women with congenital heart disease had a total of 144 pregnancies. Cardiac diagnoses were established by angiography, echocardiography, or operation. The patients were managed by the obstetric staff in conjunction with the division of cardiology adult congenital heart disease clinic. There were 50 women with acyanotic lesions, subdivided into three groups: (1) obstructive: aortic stenosis, pulmonary stenosis, and aortic coarctation; (2) left to right shunts (ventricular septal defect, atrial septal defect, patent ductus arteriosus); and (3) miscellaneous.

Twenty-four women had cyanotic lesions and were subdivided as follows: (1) tetralogy of Fallot, (2) dextrotransposition or levotransposition of the great arteries, (3) tricuspid atresia, and (4) complex cyanotic.

All groups were further classified by operative status into group A, with surgical correction, or group B, uncorrected or palliative shunt. Nine women (seven with tetralogy of Fallot, two with dextrotransposition of the great arteries) rendered acyanotic by total corrective surgery are included with the cyanotic group. Outcomes have been compared for these nine women and the patients with unrelieved cyanosis.

Table I. Outcomes for individual acyanotic lesions

Lesion	*Surgical category*	*No. of patients*	*No. of pregnancies*	*Spontaneous abortions*	*Cardiac related elective abortions*	*Other elective abortions*	*Term births*
Obstructive							
Aortic stenosis	A	3	6	—	—	1	4
	B	12	22	2	1	4	15
Pulmonic stenosis	A	2	4	1	—	—	2
	B	4	8	—	—	1	7
Coarctation	A	4	7	—	—	—	6
	B	2	2	—	2	—	—
Left to right shunt							
Ventricular septal defect	A	3	10	4	—	2	4
	B	7	11	—	—	7	3
Atrial septal defect	A	5	10	—	—	1	7
	B	2	2	—	—	—	1
Patent ductus arteriosus	B	1	3	—	3	—	—
Miscellaneous	A	2	6	—	1	2	2
	B	4	10	2	—	—	7
Total		50	101	9	7	18	58

Group A, surgical correction; group B, uncorrected or palliative shunt; CHD = congenital heart disease; RDS = respiratory distress syndrome; PDA = patent ductus arteriosus; VSD = ventricular septal defect; ASD = atrial septal defect.

Table II. Outcomes for individual cyanotic lesions

Lesion	*Surgical category*	*No. of patients*	*No. of pregnancies*	*Spontaneous abortions*	*Cardiac related elective abortions*	*Other elective abortions*	*Term births*
Tetralogy of Fallot	A	7	14	—	1	5	6
	B	2	2	—	1	—	—
Dextrotransposition of the great arteries	A	2	3	1	—	—	2
	B	2	3	1	1	—	1
Tricuspid atresia	A	—	—	—	—	—	—
	B	1	1	—	—	—	1
Patent ductus arteriosus	A	—	—	—	—	—	—
	B	1	1	—	1	—	—
Complex cyanotic lesions*	A	—	—	—	—	—	—
	B	9	19	4	2	1	5
Total		24	43	6	6	6	15

Group A, surgical correction; group B, uncorrected or palliative shunt.

*(1) Tricuspid atresia with single ventricle, pulmonary stenosis, and atrial septal defect; (2) dextrocardia, ventricular septal defect, variant transposition, ventricular inversion, and Eisenmenger's syndrome; (3) single ventricle, transposition of great arteries, and Eisenmenger's syndrome; (4) single ventricle, transposition of great arteries, and Eisenmenger's syndrome; (5) pulmonary atresia, tetralogy of Fallot, and Eisenmenger's syndrome; (6) aorticopulmonary window and Eisenmenger's syndrome; (7) patent ductus arteriosus, ventricular septal defect, and Eisenmenger's syndrome; (8) ventricular septal defect, mitral valve prolapse, and Eisenmenger's syndrome; (9) levotransposition of great arteries, dextrocardia, situs inversus, right-sided aortic arch, ventricular septal defect, and pulmonary stenosis.

†Multiple anomalies.

During pregnancy, maternal cardiac and noncardiac complications were recorded and fetal outcomes documented. All neonates were examined for congenital heart disease. Long-term assessment beyond the last complete pregnancy, for women who gave birth (as opposed to those who aborted), was obtained by telephone interviews in conjunction with their most recent assessment in the congenital heart disease clinic. During follow-up we also questioned the mothers about any changes in the cardiac status of their offspring. Statistical comparisons were done by the χ^2 method unless otherwise indicated. We considered a p value of ≤ 0.05 to be significant.

Results

Tables I and II summarize the pregnancy outcomes for individual cardiac lesions. Overall, 144 pregnancies resulted in 52 abortions and 92 continuing pregnancies,

Preterm births	*Small for gestational age babies*	*Stillbirths*	*Neonatal deaths*	*Pregnancy-induced hypertension*	*NYHA deterioration*	*Congestive heart failure*	*Remarks*
—	—	—	—	3	—	—	One incomplete pregnancy
—	1	—	1 (CHD)	2	4	2	—
1	—	—	—				
—	1	—	—	1	—	—	—
1	1	—	1 (RDS)	4	—	—	—
—	—	—	—	—	—	—	One patient underwent surgical correction after elective abortion and subsequently entered group A
—	1	—	—	—	1	—	—
1	—	1	—	1	2	—	—
1	—	—	—	—	2	—	1 Incomplete pregnancy
	—	—	—	—	—	—	1 Incomplete pregnancy (4:1 shunt)
—	—	—	—	—	—	—	—
1	—	—	—	1	—	—	1 Patient with PDA and VSD, 1 patient with ASD and VSD
1	—	—	—	—	1	1	Congenital heart block (2), Marfan's syndrome (1), dextrocardia (1)
6	4	1	2	12	10	3	—

Preterm births	*Small for gestational age babies*	*Stillbirths*	*Neonatal deaths*	*Pregnancy-induced hypertension*	*NYHA deterioration*	*Congestive heart failure*	*Remarks*
—	1	—	—	—	3	1	2 Incomplete pregnancies
1	—	—	—	—	1	—	—
—	1	—	—	1	2	1	—
—	1	—	—	—	1	1	1 Patient with Eisenmenger's syndrome underwent elective abortion
—	—	—	—	—	—	—	—
—	1	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	Patient had Eisenmenger's syndrome and underwent elective abortion
—	—	—	—	—	—	—	—
7	8	2	1†		6	4	—
8	12	2	1	1	13	7	—

of which 87 have been completed and five are still undelivered. These five are excluded from all calculations except those concerned with the incidence of abortion. Inclusion in the latter calculations is appropriate because all five pregnancies have exceeded 20 weeks of gestation. Of the 52 abortions, 15 were spontaneous and 37 elective, of which 13 were for cardiac indications (Table III). There were 31 primigravid and 43 multigravid patients. Twenty-four pregnancies occurred in women under 20 years of age, 104 in women between 20 and 30 years, and 16 in women aged 30 years or more.

Maternal complications

Noncardiac complications (Table IV). Twenty-six noncardiac complications were recorded. Half of these were pregnancy-induced hypertension that included preeclampsia and acute-on-chronic hypertension. Thus in 87 completed pregnancies the incidence of pregnancy-induced hypertension was 14.9%. This is more frequent than the occurrence in our total obstetric population during the same period of time (4.3%, $p < 0.005$). Ten of the 13 cases of pregnancy-induced hypertension occurred in women with obstructive lesions, for an incidence in this group of 27.8%. When this incidence is compared with that of the rest of the study population, the difference is significant at $p < 0.01$. Three of four patients with corrected aortic coarctation had a biscuspid valve. These three also had residual hypertension that worsened during four pregnancies. In one of these, severe preeclampsia was accompanied by abruptio placentae, renal failure, and neonatal death at 28 weeks' gestation. One woman with dextrotransposition of the great arteries, corrected by the Mustard procedure, experienced an unusual course of pre-

Table III. Elective abortions performed for cardiac indications

Lesion	*Operative status*	*NYHA class*	*No. of elective abortions*	*Comments*
Acyanotic lesions				
Aortic stenosis	B	III	1	—
Aortic coarctation	B	II	1	Had surgical repair after elective abortion, followed by 3 more pregnancies
Aortic coarctation	B	I	1	Elective abortion followed clipping of cerebral aneurysm, ruptured at 8 wk
Patent ductus arteriosus	B	III	3	—
Atrial septal defect and ventricular septal defect	B	III	1	—
Cyanotic lesions				
Tetralogy of Fallot	B	III	1	Scheduled for surgical correction of cardiac lesion
Tetralogy of Fallot, pulmonary atresia and Eisenmenger's syndrome	B	II	1	—
Patent ductus arteriosus and Eisenmenger's syndrome	B	III	1	Sterilized at same time
Dextrotransposition of great arteries and Eisenmenger's syndrome	B	III	1	—
Ventricular septal defect, mitral valve prolapse, and Eisenmenger's syndrome	B	III	1	Sterilized subsequently
Ventricular septal defect, transposition of great arteries, dextrocardia, and Eisenmenger's syndrome	B	III	1	—
Total			13	

Table IV. Obstetric and other noncardiac complications

Complication	*No. of episodes*
Pregnancy-induced hypertension	13
Gestational diabetes	3
Anemia (hemoglobin level < 100 gm/L)	2
Placenta previa	2
Vasa praevia	1
Abruptio placentae with renal failure	1
Postpartum hemorrhage	1
Splenic torsion and infarction	1
Ruptured cerebral aneurysm	1
Incompetent cervix	1
Total	26

eclampsia wherein progressive proteinuria (reaching 3.0 gm/24 hours) was unaccompanied by hypertension until 1 day before delivery, when the blood pressure rose to 150/95 mm Hg. The preeclampsia resolved in the early puerperium.

Delayed postpartum hemorrhage occurred in a woman with Eisenmenger's syndrome who was on a regimen of prophylactic postdelivery heparin therapy. The hemoglobin level fell from 183 to 114 gm/L. She required packed cells, fresh-frozen plasma, emergency curettage for removal of a small placental fragment, and finally estrogen therapy before bleeding ceased.

Cardiac complications (Table V). All patients were New York Heart Association (NYHA) functional class I or II at the outset, except for eight women with class III symptoms who underwent elective abortion. Of the 87 completed pregnancies, NYHA deterioration occurred in 23 (26.4%) and congestive heart failure in 10 (11.5%). In four patients, supraventricular arrhythmias developed; all were medically controlled. Functional deterioration occurred in 13 of 23 pregnancies (56.5%) in the cyanotic group compared with 10 of 64 (15.6%) in the acyanotic group ($p < 0.005$). Similarly, congestive heart failure was more frequent ($p < 0.005$) in the cyanotic group (seven of 23, 30.4%) than in the acyanotic group (three of 64, 4.7%). In most instances the degree of functional deterioration was mild to moderate, with three exceptions. The first, a women with Marfan's syndrome, had experienced two uncomplicated pregnancies. In her third pregnancy, at 38 weeks' gestation, she suffered aortic dissection that extended to the coronary arteries. Cardiac arrest ensued. She was resuscitated and underwent emergency cesarean delivery followed by immediate graft repair to the damaged arteries. The second patient was a woman with aorticopulmonary window and Eisenmenger's syndrome. Five hours after planned cesarean delivery for chronic fetal distress, she became hypotensive and cyanosis deepened. She experienced a cardiac arrest and died. Postmortem examination revealed blood in all serous cavities, leading to the speculation that a coagulopathy had contributed to her death. She was not receiving anticoagulants at the time.

In the third case, a women with severe aortic stenosis

Table V. Cardiac complications in continuing pregnancies

Complication	*Acyanotic lesions*		*p Value*	*Cyanotic lesions*	
	No.	*%*		*No.*	*%*
NYHA deterioration	10/64	15.6	<0.005	13/23	56.5
Congestive heart failure	3/64	4.7	<0.005	7/23	30.4
Arrhythmias	2/64	4.7	—	2/23	8.7
Infective endocarditis	2/64	3.1	—	—	—
Aortic dissection	1/64	1.6	—	—	—

developed left ventricular failure at 30 weeks' gestation. At 38 weeks she had a fever of unknown origin that resolved with antibiotic treatment. One week later, while she was receiving antibiotics, she had a normal vaginal delivery. One month later she was readmitted to the hospital with infective endocarditis and recurrent cardiac failure. She required aortic valve replacement 5 months post partum.

One patient had postpartum infective endocarditis. A primigravida with corrected atrial septal defect and ventricular septal defect, she had not received preoperative antibiotics before emergency cesarean section for ruptured vasa praevia. The infection was treated with antibiotics and gradually resolved.

Maternal follow-up (Table VI). Fifty of the 74 women gave birth at least once. Twenty-six of 34 women with acyanotic lesions (76.5%) have been followed up. Of the 16 women with cyanotic lesions who were delivered, one died post partum. Ten of the remaining 15 women (66.7%) have been followed up. The duration of follow-up ranges from 3 to 112 months (average, 30 months). The patients were evaluated with respect to cardiac developments such as change in functional status and the need for further operation. Only five of 26 women (19.2%) with acyanotic disorders had important sequelae, compared with 9 of 10 women (90%) in the cyanotic group ($p < 0.005$). In the acyanotic group, two patients with atrial septal defect were scheduled for operation; one had an aortic valve replacement and another experienced NYHA functional deterioration. The patient with Marfan's syndrome was readmitted 3 months post partum with dissection of the abdominal aorta. In the cyanotic group, one patient with corrected dextrotransposition of the great arteries died of cardiac arrhythmia 18 months post partum. Two patients are awaiting Fontan procedures. Another developed infective endocarditis and, as well, recently underwent operation for repair of ventricular septal defect and conduit bypass of pulmonary artery stenosis; five have experienced mild to moderate NYHA class deterioration. In one of these, a woman with uncorrected tetralogy of Fallot, Eisenmenger's syndrome has developed.

Two patients, one with a porcine aortic valve (congenital atrial septal defect) and one with corrected tetralogy of Fallot, became pregnant again.

Table VI. Follow-up data on patients who have been delivered of infants

Event	*Acyanotic group (N = 26/34, 76.5%)*	*Cyanotic group (N = 10/15, 66.7%)*
Deterioration in NYHA status	1/26	5/10
Infective endocarditis	—	1/10
Pulmonary hypertension	—	1/10
Abdominal aorta dissection	1/26	—
Cardiac surgery planned or performed	3/26	3/10
Died	—	1/10
Currently pregnant again	1/26	1/10

Duration of follow-up: <1 year, 4 (patients); 1 to 2 years, 18; 2 to 4 years, 8; >4 years, 6. Range of follow-up, 3 to 112 months (average, 30 months).

Fetal outcome (Table VII). There were 37 elective abortions. Thus 107 of 144 pregnancies have followed a natural course, including five undelivered pregnancies that (at this writing) have exceeded 20 weeks' gestation. In the acyanotic group, nine of 76 (11.9%) spontaneously aborted, compared with six of 31 (19.4%) in the cyanotic group. The perinatal mortality in the acyanotic group was 4.7% (three of 64) compared with 3.0% (three of 23) in the cyanotic group. The overall mean birth weight for infants in the acyanotic group was 3300 ± 600 gm compared with 2400 ± 800 gm in the cyanotic group ($p < 0.005$, Student's t test). Infants who were small for gestational age (neonatal weight less than tenth percentile for gestational age) were more frequent in the cyanotic group ($p < 0.005$) as were preterm births ($p < 0.01$). One neonate (1.1%) had congenital heart disease. Born to a mother with atrial septal defect, this infant died at age 4 weeks of cardiac failure resulting from coarctation. Two of three infants born to the patient with Marfan's syndrome have inherited this disorder.

Labor and delivery (Table VIII). The mean primary cesarean delivery rate was 21.8%, compared with the overall mean rate of 16% at the Toronto General Hospital during the same period. Although dystocia was

Table VII. Fetal outcome*

Complication	Acyanotic group		p Value	Cyanotic group	
	No.	%		No.	%
Spontaneous abortion	9/76	11.9	—	6/31	19.4
Perinatal mortality	3/64	4.7	—	3/23	13.0
Small for gestational age	4/64	6.3	<0.005	12/23	52.2
Preterm birth	6/64	9.4	<0.01	8/23	34.8
Congenital heart disease	1/64	1.6	—	—	—
Other anomalies	—	—	—	1/23	4.3
Mean birth weight (gm)	3300 ± 600	—	<0.005	2400 ± 800	—

*Five pregnancies are undelivered but have exceeded 20 weeks' gestation.

Table VIII. Method of delivery

Method	No.	%
Vaginal		
Spontaneous	36	
Low forceps	16	
Midforceps	8	
Assisted breech	2	
Total	62	71.2
Cesarean		
Primary*	19	21.8
Repeat	6	6.9
Total	25	28.7
Indications for primary cesarean section		
Preeclampsia	4	
Dystocia	4	
Acute fetal distress	3	
Chronic fetal distress	3	
Placenta previa	2	
Vasa praevia	1	
Preterm breech	1	
Aortic dissection	1	
Total	19	

*Primary cesarean section rate for hospital population during the same period = 16%.

Table IX. Outcomes in cases of Eisenmenger's syndrome

	Number	%
Patients	9	100
Pregnancies	19	100
Spontaneous abortions	4	21.1
Therapeutic abortions	5	26.3
Term births	4	21.1
Preterm births	6	31.6
Small for gestational age infants	6	31.6
Stillbirths	1	5.3
Neonatal deaths	1	5.3
Pregnancy-induced hypertension	0	0
NYHA deterioration	5	26.3
Congestive heart failure	3	15.8
Maternal death	1	5.3

the commonest indication in the general population, it was no more frequently a reason for performing cesarean section than fetal distress (acute and chronic) or preeclampsia (with conditions unfavorable for induction of labor) in the study group.

Eisenmenger's syndrome (Table IX). Nine patients had a total of 19 pregnancies. Maternal and fetal complications were high in this group, which included the only maternal death. In particular, compared with incidences in the remaining patients in this study, preterm births and small for gestational age infants were significantly more frequent ($p < 0.005$).

Group A (surgical correction) versus group B (uncorrected or palliative shunt) (Table X). Groups A and B were compared with respect to cardiovascular and fetal outcomes in both the acyanotic and cyanotic groups. This assessment was not possible for each individual lesion because some patients (with uncorrected patent ductus arteriosus, uncorrected aortic coarctation) invariably underwent elective abortion and others (complex cyanotic) were confined to group B. Overall, the clearest demonstrable benefits derived from corrective surgery were seen in the cyanotic category where group A patients had a lower incidence of spontaneous abortion ($p < 0.01$) and small for gestational age infants ($p < 0.02$) and a greater achievement of term births ($p < 0.01$) than group B.

Comment

In women with congenital heart disease, pregnancy may be affected by (1) the functional cardiac status, (2) the anatomic diagnosis, (3) pulmonary hypertension, and (4) the type of operation and degree of residual impairment, if any.

Functional cardiac status. Maternal functional status is known to strongly influence outcome. Although only 20% of patients in this study are NYHA class III or IV, they account for 85% of maternal deaths.[3] Whittemore et al.[2] observed significant cardiac complications in 10% of women with class I or II symptoms, compared with 30% in women with class III or IV symptoms. Accordingly, in our unit women with class III or IV symptoms have been offered, and have accepted, pregnancy termination. Thus continuing pregnancies in this study were limited to women classified as NYHA I or II at the outset.

Reliance on functional status alone may, however, be

Table X. Outcomes for group A (surgical correction) versus group B (uncorrected or palliative shunt)*

Event	Group A		p Value	Group B	
	No.	%		No.	%
Acyanotic lesions					
Spontaneous abortions	5/34	14.7	—	4/39	10.3
Small for gestational age infants	2/29	6.9	—	2/35	5.7
Term births	25/29	86.2	—	33/35	94.3
NYHA deterioration	3/29	10.3	—	7/35	20.0
Congestive heart failure	0/29	0	—	3/35	8.6
Cyanotic lesions					
Spontaneous abortions	1/9	11.1	<.01	5/20	25.0
Small for gestational age infants	2/8	25.0	<.02	10/15	66.7
Term births	8/8	100.0	<.01	7/15	46.7
NYHA deterioration	5/8	62.3	—	8/15	53.3
Congestive heart failure	2/8	25.0	—	5/15	33.3

*Elective abortions excluded; undelivered pregnancies (n = 5) included only in calculations for spontaneous abortion.

misleading. Sudden and profound deterioration can result from anatomic deformities, hemodynamic alterations, or even therapeutic measures. Examples in our experience included aortic dissection, postpartum worsening of a right to left shunt, and delayed postpartum hemorrhage during treatment with anticoagulants.

Anatomic diagnosis. In the absence of pulmonary hypertension or large left to right shunts, women with patent ductus arteriosus, atrial septal defect, and ventricular septal defect usually tolerate pregnancy well, although uncorrected atrial septal defect and ventricular septal defect have each been associated with a maternal mortality of 5.5%[4] and fetal loss rates of 10% to 25%.[5] Maternal deaths have resulted from heart failure, infective endocarditis, or shunt reversal (after arterial hypotension or acute pulmonary hypertension secondary to pulmonic embolism). In the present study, 21 continuing pregnancies in women with left to right shunts resulted in four spontaneous abortions and 17 births, two of which were preterm. Two women with uncorrected atrial septal defects became pregnant while awaiting admission for corrective cardiac surgery. The first of these, with a 2.8:1 shunt, experienced a normal delivery at term. The second, presently 32 weeks pregnant, has a 4:1 shunt. The fetus is developing normally and NYHA status is stable at class II.

Women with pulmonic stenosis usually tolerate the increased right ventricular load seen in pregnancy. Although older data suggested an 8% incidence of cardiac failure,[4] more recently this complication occurred in only 2.8%.[2] Fetal losses vary but have been reported in as high as 20% of pregnancies.[5] We observed one spontaneous abortion, 10 live births, and no cases of cardiac failure.

Congenital aortic stenosis during pregnancy carries an increased risk of left ventricular failure, sudden death, and, particularly in the presence of a bicuspid valve, infective endocarditis.[6] Arias and Pineda[7] reported maternal and fetal mortalities of 17% and 32%, respectively, but their data included rheumatic valvular disease. Neilson et al.[8] reported no complications during 10 pregnancies in women with congenital aortic stenosis. In the series of Whittemore et al.,[2] 18 pregnancies occurred in women with left ventricular outflow obstruction and only fair or poor function. There were five instances of congestive heart failure and six of increased arterial blood pressure. Their data did not distinguish between aortic stenosis and aortic coarctation. We had 15 patients with congenital aortic stenosis. Excluding elective abortions, one of which was performed for class III symptoms, 21 pregnancies resulted in two spontaneous abortions, 19 term births, one neonatal death from aortic coarctation, two cases of cardiac failure, and five instances of pregnancy-induced hypertension.

In a literature review, Goodwin[9] reported a maternal mortality rate of 3.5% in pregnancies with uncorrected coarctation. Deaths usually resulted from aortic rupture or dissection (80% before labor and unrelated to the height of blood pressure), heart failure, cerebral hemorrhage, or infective endocarditis. Deal and Wooley[10] collected data on 185 pregnancies with uncorrected aortic coarctation in which no maternal deaths occurred. Blood pressure changes during pregnancy were characteristic of normal patients. Both studies noted a high fetal loss rate, possibly from diminished uteroplacental blood supply. Both of our patients with uncorrected aortic coarctation underwent elective abortion, one for severe systolic hypertension (>200 mm Hg) and the other after surgical clipping of a ruptured cerebral aneurysm. In the corrected group, four of seven pregnancies demonstrated pregnancy-induced hypertension, including one severe case with renal failure and neonatal death. These hypertensive changes contrast with the findings reported above.[10]

In young women, 50% of aortic dissections occur during pregnancy.[6] Szekely and Julian[6] described 15

cases, nine of which occurred in women with Marfan's syndrome and four in women with signs suggestive of this disorder. In susceptible women, pregnancy heightens the risk of aortic dissection or rupture by hemodynamic effects (widened pulse pressure, increased stroke volume) instead of by altered aortic histologic characteristics as was previously thought.[11, 12] Marfan's syndrome and pregnancy is a risky combination because (1) 50% of offspring inherit the disorder, (2) there is an increased risk of aortic dissection, and (3) there is usually a shortened life span (half normal), thus compromising the woman's ability to raise her child.[5] Reporting only one death in 26 pregnant women with Marfan's syndrome, Peyritz[13] concluded that the risk could be minimized by preconceptional echocardiography. He considered the low-risk patient to have minimal, if any, aortic root dilation and no aortic regurgitation or significant mitral valve dysfunction. Our own experience with one patient, however, exemplifies the risks stated above, in that two of her three children have Marfan's syndrome, the aorta of this patient dissected during pregnancy, and her present prognosis is grave because recently the abdominal aorta dissected.

Tetralogy of Fallot is the most common cyanotic congenital heart disease in adults and children today.[14] Uncorrected, it carries a 4% risk of maternal death and a 30% fetal mortality from hypoxia.[4] In pregnancy, the fall in peripheral vascular resistance increases the right to left shunt. Recurrent syncope, a hematocrit above 60, an arterial oxygen saturation below 80, and a right ventricular pressure above 120 mm Hg are poor prognostic factors.[6] Hypotension from blood loss or epidural anesthesia during labor and delivery can precipitate increased cyanosis and circulatory collapse. Two women in our series had uncorrected tetralogy of Fallot resulting in one elective abortion and one preterm birth. All six continuing pregnancies in women with corrected tetralogy of Fallot went to term with only one small for gestational age infant. Congestive heart failure occurred once in this group.

Data on other cyanotic lesions during pregnancy are scant. Collins et al.[15] described three cases of tricuspid atresia. The first experienced three spontaneous abortions, the second died in late pregnancy of massive hemoptysis, and the third was delivered prematurely of a 580 gm infant who died of immaturity in a pregnancy complicated by heart failure and pulmonary and cerebral emboli. We had one patient with tricuspid atresia and another with tricuspid atresia and a single ventricle. The former, after an unremarkable pregnancy, had a term small for gestational age infant. The latter had an uneventful maternal course but was prematurely delivered of a 1490 gm small for gestational age infant.

Six cases of a single ventricle and pregnancy have been reported and were reviewed by Stiller et al.,[16] who cited the following: maternal mortality, 16%; small for gestational age, 43%; preterm labor, 43%; cesarean deliveries, 70%. We had three patients with a single ventricle who had a total of five pregnancies and observed: maternal deaths, 0%; small for gestational age, 60%; preterm labor, 40%; and cesarean deliveries, 60%.

Neilson et al.[8] reported one case of a patient with uncorrected transposition of the great arteries who experienced two spontaneous abortions and one preterm neonatal death. Two of our patients had uncorrected dextrotransposition of the great arteries, one with a ventricular septal defect and one with Eisenmenger's syndrome. The former had one spontaneous abortion and one small for gestational age but term infant. She experienced congestive heart failure in the latter pregnancy. The other patient had one elective abortion. Two women with dextrotransposition of the great arteries corrected by the Mustard operation had a total of three pregnancies, which included one spontaneous abortion, one small for gestational age infant in a term pregnancy, and one normal term birth. Pregnancy-induced hypertension occurred in one patient and congestive heart failure in the other.

Pulmonary hypertension. This condition, as seen in Eisenmenger's syndrome, is associated with 35% maternal mortality, a 55% prematurity rate, a 30% small for gestational age rate, and 28% perinatal mortality.[14] The pathophysiologic cause of death is likely an increase in right to left shunting after a decrease in systemic vascular resistance from blood loss or epidural-related hypotension. These events favor increased cyanosis and hypoxia possibly compounded by pulmonary arterial stasis and thrombosis during the hypercoagulable postpartum state.[17] For patients in labor, Midwall et al.[18] demonstrated beneficial effects of high concentrations of oxygen that consisted of a fall in pulmonary arterial pressure and an increase in the ratio of pulmonary-to-systemic blood flow and the arterial oxygen saturation.

The use of heparin during the postpartum period to prevent intra-arterial pulmonary thromboses has not been shown to prevent deterioration and may indeed be hazardous,[17] as was the case in one of our patients who experienced a severe postpartum hemorrhage. In our experience, Eisenmenger's syndrome was associated with a high complication rate including one maternal death. However, this is a relatively low maternal mortality rate compared with those of other series[14] and may be attributed to some selection bias in that several of our more severely affected patients have accepted either sterilization or elective abortion (Table III).

Surgical correction. Because many congenital heart defects are now surgically correctable, a new population of women have reached adulthood and achieved pregnancy. Patients with corrected atrial septal defects, ven-

tricular septal defects, or simple coarctations appear to be undertaking pregnancy with no added risk.[14] Similarly, providing no residual impairment exists, women with corrected tetralogy of Fallot have excellent outcomes. Singh et al.[19] reviewed 40 pregnancies in 27 women with completely corrected tetralogy of Fallot and no residua. There were no abortions, premature births, small for gestational age infants, or serious cardiac complications. One neonate had pulmonary atresia. Whittemore et al.[2] observed that live births occurred in only 42% of pregnancies with unrelieved cyanosis. With palliative shunts there was a 72% live-birth rate but the infants were usually growth retarded. Total surgical correction was accompanied by a 78% live-birth rate with normally grown infants, results similar to our own.

Despite surgical advances, however, patients who have undergone palliative and corrective procedures continue to pose problems. Twenty-five years after palliative operation only 35% of patients are not disabled. A total of 10% to 25% of patients exhibit resting hypertension after correction of coarctation.[20] Other problems exist such as patch leaks, postvalvulotomy regurgitation, conducting system injuries, and blunted ventricular performance due to the effects of chronic volume and pressure overload, intraoperative ischemia, and scar formation.[21] Some of these problems were evident in the present study. Examples included postcoarctectomy hypertension, congestive heart failure in two women with corrected tetralogy of Fallot, and, possibly, a blunted pressor response in the patient with dextrotransposition of the great arteries, whose preeclampsia manifested primarily as proteinuria. This patient also experienced a supraventricular arrhythmia, a relatively common sequel to the Mustard procedure.

Congenital heart disease in neonates. Our incidence of 1.1% is low compared with the commonly quoted rates of 3% to 5% (and as high as 14%).[2] This may relate to the fact that in the absence of positive findings, our infants only underwent clinical evaluation. As well, only 73.5% of patients received long-term follow-up. In two of our telephone interviews, the mothers reported "murmurs" in the children, which had spontaneously disappeared. The high incidence quoted by Whittemore et al.[2] is undoubtedly related to a diligent search for cardiac lesions. In their series the most common defect was ventricular septal defect, and by age 3 years seven of 18 had spontaneously closed.

Therapeutic considerations. Before becoming pregnant, our patients with congenital heart disease are seen in the adult congenital heart disease clinic and the high-risk pregnancy unit. They have a careful clinical examination, electrocardiogram, chest x-ray examination, and in most instances echocardiographic evaluation. Most patients will have already undergone cardiac catheterization and many will have had surgical correction. They are apprised of the maternal and fetal risks associated with pregnancy. Cardiac surgery, where indicated (such as large left to right shunts, uncorrected tetralogy of Fallot, or aortic coarctation) should be performed before pregnancy is considered. Because the physical condition of the mother will, at least in part, influence her ability to perform the tasks of motherhood, the long-term prognosis is relevant to prepregnancy counseling and should be discussed in a sensitive but informative manner. In our study, cyanotic patients on the whole experienced more frequent cardiac sequelae ($p < 0.001$); however, other conditions such as Marfan's syndrome or symptomatic aortic stenosis carry an unfavorable prognosis. Thus each case must be individually evaluated.

Our current policy is to recommend against pregnancy or to offer elective abortion to patients with NYHA class III or IV symptoms and those with pulmonary hypertension. Patients with Marfan's syndrome should be similarly advised on both maternal and fetal grounds. Although the data on aortic coarctation are less clear, we believe that pregnancy should be undertaken only after corrective surgery and in the absence of significant associated defects such as bicuspid aortic valve or a gradient of >40 mm Hg across the coarctation. High-risk factors for patients with unrelieved cyanosis are discussed earlier with the data on tetralogy of Fallot. These patients should be told of their high predilection for delivering preterm small for gestational age infants.

Once pregnant, patients with congenital heart disease require a multidisciplinary approach involving the cardiologist, obstetrician, cardiac anesthetist, and neonatologist. All patients require increased rest periods, more frequent prenatal visits, and immediate treatment of acute illness such as respiratory tract infection or congestive heart failure. Iron therapy for prevention of anemia is important. Tailoring the management to specific needs is also essential. For example, patients with obstructive lesions should be closely monitored for pregnancy-induced hypertension, cyanotic patients for evidence of fetal dysmaturity, and those with Marfan's syndrome for signs of dissection. Similarly, while an agent such as propranolol might reduce the risk of aortic dissection in Marfan's syndrome, it would be contraindicated in women with significant aortic stenosis who must rely on an increased heart rate to achieve the cardiac output required during pregnancy. Fetal echocardiography, by allowing prenatal detection of a cardiac lesion, might expedite neonatal medical and surgical intervention when indicated.

Whenever possible we aim for vaginal delivery, reducing pain by controlled epidural anesthesia and minimizing bearing-down effects by shortening the second

stage. Other intrapartum measures include administration of oxygen, antibiotic prophylaxis (after delivery as well), and appropriate hemodynamic and electrocardiographic monitoring. Assessment of oxygen saturation and changes in shunt flow by arterial blood gases and/or finger oximetry are essential in patients with shunts. Hypotension induced by blood loss or epidural anesthesia must be rigorously avoided in women with shunts or significant aortic stenosis.

Postpartum patients at high risk, such as those with Eisenmenger's syndrome or critical aortic stenosis, must be initially managed in an intensive care unit. In the postnatal period, the relative risks of birth control measures, sterilization procedures, and future pregnancies should be discussed with the patient.

REFERENCES

1. Cannell DE, Vernon CP. Congenital heart disease and pregnancy. AM J OBSTET GYNECOL 1963;85:744.
2. Whittemore RH, Hobbins JC, Engle MA. Pregnancy and its outcome in women with and without surgical treatment of congenital disease. Am J Cardiol 1982;50:641.
3. Nesbitt R Jr. Coincidental medical disorders complicating pregnancy. Cardiovascular diseases. In: Danforth DN, ed. Obstetrics and gynecology. 3d ed. New York: Harper & Row, 1977:430-7.
4. Mendelson CL. Cardiac disease in pregnancy. Philadelphia: FA Davis, 1960.
5. McAnulty JH, Metcalfe J, Ueland K. Cardiovascular disease. In: Burrow GN, Ferris TF. Medical complications of pregnancy. Toronto: WB Saunders, 1982:135.
6. Szekely P, Julian D. Heart disease and pregnancy. Curr Probl Cardiol 1979;4:6.
7. Arias F, Pineda J. Aortic stenosis and pregnancy. J Reprod Med 1978;20:229-32.
8. Neilson G, Galea EG, Blunt A. Congenital heart disease and pregnancy. Med J Aust 1970;1:1086.
9. Goodwin JF. Pregnancy and coarctation of the aorta. Clin Obstet Gynecol 1961;4:645.
10. Deal K, Wooley CF. Coarctation of the aorta and pregnancy. Ann Intern Med 1973;78:706-10.
11. Manalo-Estrella P, Barker AE. Histopathologic findings in human aortic media associated with pregnancy: a study of 16 cases. Arch Pathol 1967;83:336-41.
12. Cavanzo FJ, Taylor HB. Effects of pregnancy on the human aorta and its relationship to dissecting aneurysms. AM J OBSTET GYNECOL 1969;105:567.
13. Peyritz RE. Maternal and fetal complications of pregnancy in the Marfan syndrome. Am J Med 1981;46:784.
14. Cobb T, Gleicher N, Elkayam U. Congenital heart disease in pregnancy. In: Elkayam U, Gleicher N. Cardiac disease and pregnancy. New York: Alan R Liss, 1982:61.
15. Collins ML, Leal J, Thompson NJ. Tricuspid atresia and pregnancy. Obstet Gynecol 1982;60:641.
16. Stiller RJ, Vintzileos AM, Nochinson DJ, et al. Single ventricle in pregnancy: case report and review of the literature. Obstet Gynecol 1984;64:18S.
17. Pitts JA, Crosby WM, Basta LL. Eisenmenger's syndrome in pregnancy. Am Heart J 1977;93:321.
18. Midwall J, Jaffin H, Herrman MV, Kupersmith J. Shunt flow and pulmonary haemodynamics during labour and delivery in the Eisenmenger's syndrome. Am J Cardiol 1978;42:299.
19. Singh H, Bolton PJ, Oakley CM. Pregnancy after surgical correction of tetralogy of Fallot. Br Med J 1982;285:168.
20. McNamara DG, Latson LA. Long-term follow-up of patients with malformations for which definite surgical repair has been available for 25 years or more. Am J Cardiol 1982;50:560.
21. Graham TP. Ventricular performance in adults after operation for congenital heart disease. Am J Cardiol 1982; 50:612.

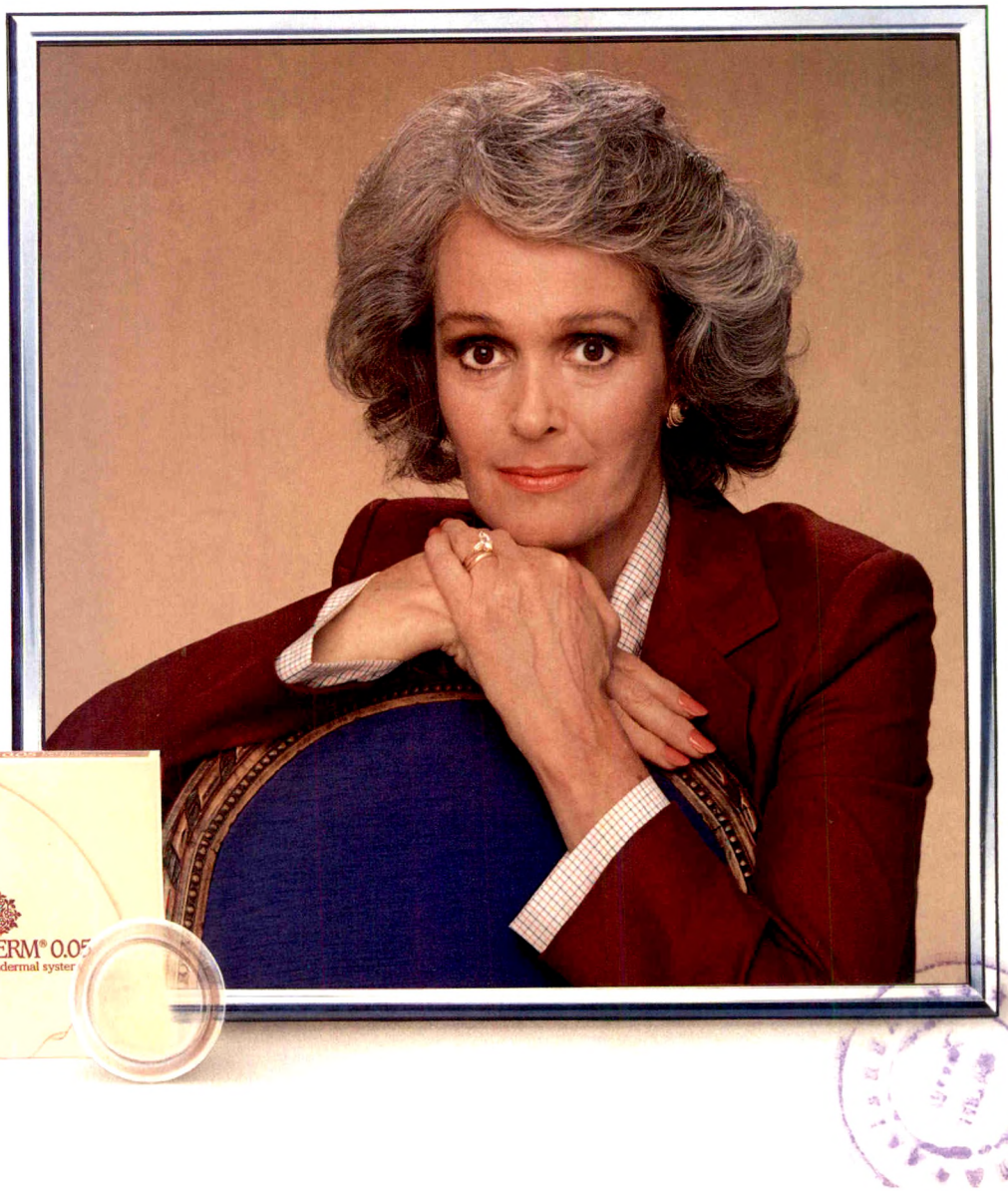




Now

The next generation of estrogen replacement therapy...

transdermal delivery

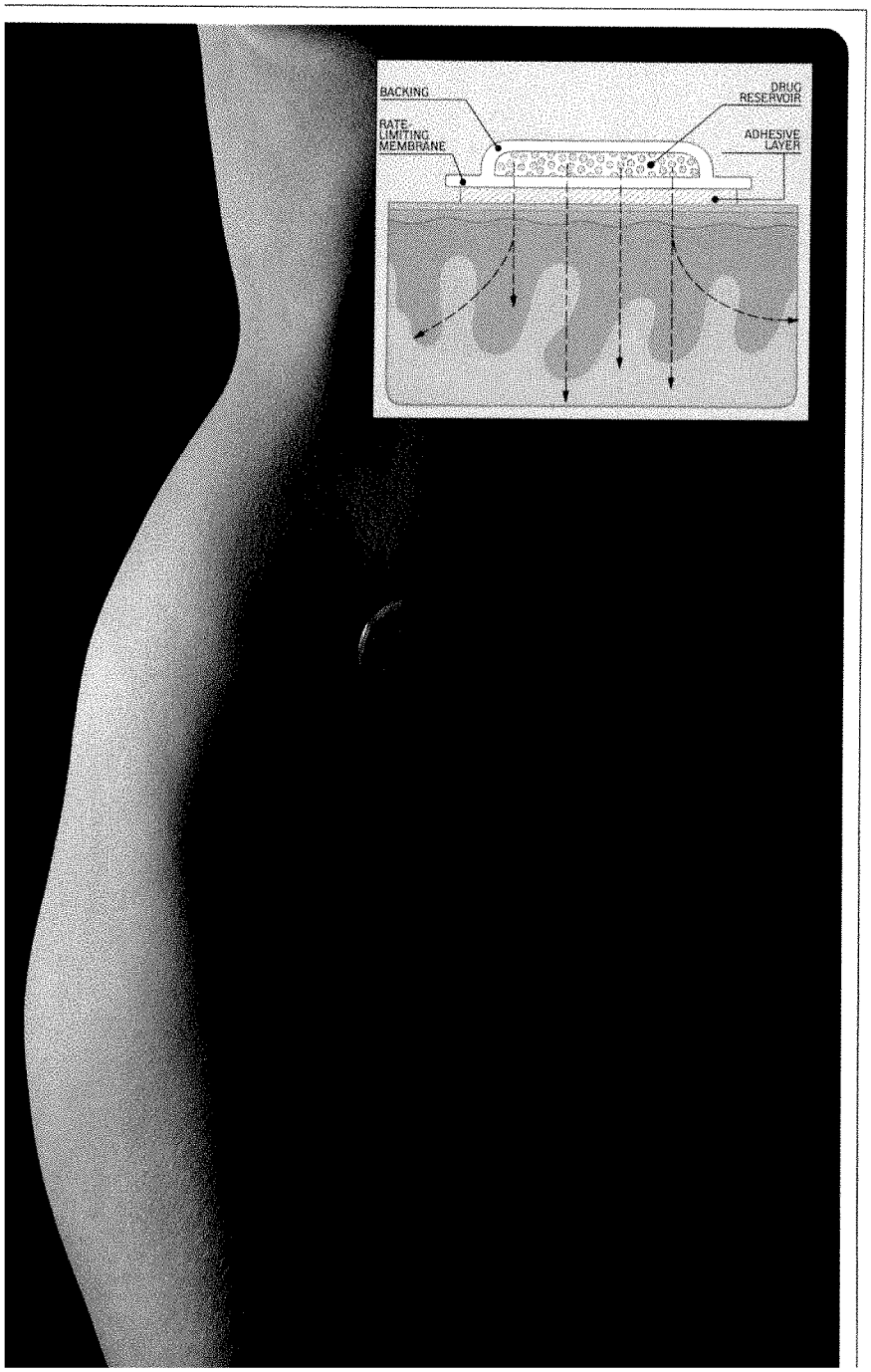


NEW

ESTRADERM®

estradiol transdermal syster

Combines the advantages of transdermal delivery...



A specially designed transdermal delivery system

Providing the natural ovarian estroge (17-beta estradiol) directly to the bloodstream and target organs; with no first-pass liver metabolism, permi use of extremely low dosage

Effectively controls estrogen deficiency symptoms

Steady-state relief of hot flashes, oth menopausal symptoms associated wi hot flashes, and vaginal discomfort

Well tolerated

Very low incidence of systemic side effects...few local problems of consequence

Easy to use; transdermal delivery preferred 3:1 by patients over tablets[1]

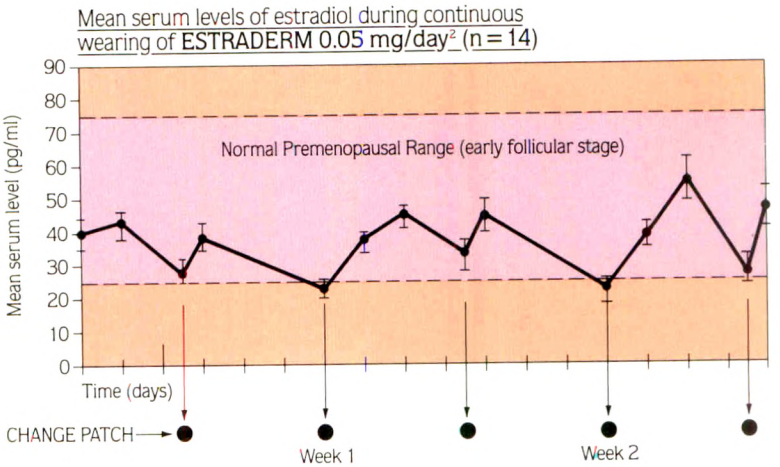
Usual starting dose: a 0.05-mg syste changed twice weekly

C I B A

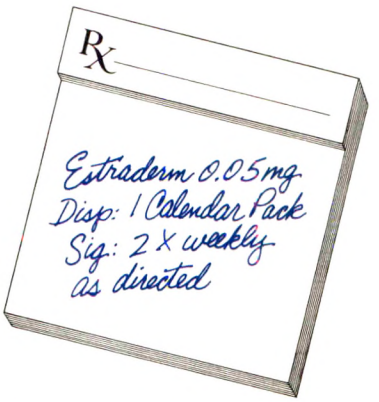
/ith the benefits of strogen replacement

:stores and maintains premenopausal tradiol serum levels

:tores estradiol levels to near-normal premenopausal ranges
chieves a physiologic 1:1 ratio of estradiol to estrone







ailable in dose-reminder calendar packs of eight
tches—0.05 mg/0.1 mg

: I B A
lping today's women live healthier lives

References:

1. Data on file, CIBA Pharmaceutical Company.
2. Powers MS, et al: Pharmacokinetics and pharmacodynamics of transdermal dosage forms of 17 β-estradiol: Comparison with conventional oral estrogens used for hormone replacement. Am J Obstet Gynecol 1985; 152:1099–1106.

Please see next page
for Brief Summary of Prescribing Information.

ESTRADERM®

estradiol transdermal system

Continuous delivery for twice-weekly application.
BRIEF SUMMARY OF PRESCRIBING INFORMATION.
PLEASE SEE FULL PRESCRIBINC INFORMATION.

ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than 1 year. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer-reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5-13.9 times greater than in nonusers. The risk appears to depend both on duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.

The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that women who had been exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than four per 1000 exposures. Furthermore, a high percentage of such exposed women (30-90%) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data on the use of other estrogens are not available, it cannot be presumed they would not induce similar changes.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb-reduction defects. One case control study estimated a 4.7-fold increased risk of limb-reduction defects in infants who had been exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses.

If Estraderm is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus and of the advisability of continuation of the pregnancy.

INDICATIONS AND USAGE

Estraderm is indicated for the treatment of the following: moderate-to-severe vasomotor symptoms associated with menopause; female hypogonadism; female castration; primary ovarian failure; and atrophic conditions caused by deficient endogenous estrogen production, such as atrophic vaginitis and kraurosis vulvae.

Studies of the addition of a progestin for seven or more days of a cycle of estrogen administration have reported a lowered incidence of endometrial hyperplasia. Morphological and biochemical studies of endometrium suggest that 12 to 13 days of progestin are needed to provide maximal maturation of the endometrium and to eliminate any hyperplastic changes. Whether this will provide protection from endometrial carcinoma has not been clearly established. There are possible additional risks that may be associated with the inclusion of progestin in estrogen replacement regimens. The potential risks include adverse effects on carbohydrate and lipid metabolism. The choice of progestin and dosage may be important in minimizing these adverse effects.

CONTRAINDICATIONS

Estrogens should not be used in women or men with any of the following conditions:

1. known or suspected cancer of the breast;
2. known or suspected estrogen-dependent neoplasia;
3. known or suspected pregnancy (see Boxed Warning);
4. undiagnosed abnormal genital bleeding;
5. active thrombophlebitis or thromboembolic disorders;
6. history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use.

WARNINGS

1. Induction of Malignant Neoplasms. Long-term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. There are now reports that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning.)

At the present time, there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of breast cancer, although a recent long-term follow-up of a single physician's practice has raised this possibility. Because of the animal data, there is a need for caution in prescribing estrogens for women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms.

2. Gallbladder Disease. A recent study has reported a two- to threefold increase in the risk of surgically confirmed gallbladder disease in postmenopausal women receiving oral estrogens, similar to the twofold increase previously noted in users of oral contraceptives.

3. Effects Similar to Those Caused by Estrogen-Progestogen Oral Contraceptives. There are several serious adverse effects of oral contraceptives and other high-dose oral estrogen treatments, most of which have not, up to now, been documented as consequences of postmenopausal estrogen replacement therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women.

a. Thromboembolic Disease. It is now well established that users of oral contraceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least four weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization.

While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found, this does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore, estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be used in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

b. Hepatic Adenoma. Benign hepatic adenomas have been associated with the use of oral contraceptives. Although benign and rare, these tumors may rupture and cause death from intra-abdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations, but they should be considered if abdominal pain and tenderness, abdominal mass, or hypovolemic shock occurs in patients receiving estrogen. Hepatocellular carcinoma has also been reported in women taking estrogen-containing oral contraceptives. The causal relationship of this malignancy to these drugs is not known.

c. Elevated Blood Pressure. Women using oral contraceptives sometimes experience increased blood pressure which, in most cases, returns to normal upon discontinuing the drug. There is now a report that this may occur with use of oral estrogens in the menopause and blood pressure should be monitored with estrogen use, especially if high doses are used. Ethinyl estradiol and conjugated estrogens have been shown to increase renin substrate. In contrast to these oral estrogens, transdermally administered estradiol does not affect renin substrate.

d. Glucose Tolerance. A worsening of glucose tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen.

4. Hypercalcemia. Administration of high doses of estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases. If hypercalcemia occurs, use of the drug should be stopped and appropriate measures should be taken to reduce the serum calcium level.

PRECAUTIONS

General

1. A complete medical and family history should be taken before initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, as well as a cervical Papanicolaou test. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed.
2. Because estrogens may cause some degree of fluid retention, careful observation is required when conditions that might be influenced by this factor are present (e.g., asthma, epilepsy, migraine, and cardiac or renal dysfunction).
3. Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as uterine bleeding, mastodynia, etc.
4. Prolonged administration of unopposed estrogen therapy has been reported to increase the risk of endometrial hyperplasia in some patients. Estrogens should be used with caution in patients who have or have had endometriosis.
5. Oral contraceptives appear to be associated with an increased incidence of mental depression. Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.
6. Preexisting uterine leiomyomata may increase in size during prolonged estrogen use. If this occurs, estrogen therapy should be discontinued while the cause is investigated.
7. In patients with a history of jaundice during pregnancy, there is an increased risk that jaundice will recur with the use of estrogen-containing oral contraceptives. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated.
8. Estrogens may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients.
9. Because the prolonged use of estrogens influences the metabolism of calcium and phosphorus, estrogens should be used with caution in patients with metabolic bone diseases associated with hypercalcemia and in patients with renal insufficiency.

Drug/Laboratory Test Interactions

The results of certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The following changes have been observed with large doses of oral estrogen:

1. increased sulfobromophthalein retention;
2. increased prothrombin time; increased factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability;
3. increased thyroxine-binding globulin (TBG) leading to increased circulating total thyroid hormone (T_4) as measured by column or radioimmunoassay; free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered; TBG was not affected in clinical trials of Estraderm;
4. reduced response to the metyrapone test;
5. reduced serum folate concentration;
6. increased serum triglyceride and phospholipid concentration, and decreased pregnanediol excretion.

The pathologist should be informed that the patient is receiving estrogen therapy when relevant specimens are submitted.

Carcinogenesis, Mutagenesis, Impairment of Fertility

See **WARNINGS** and Boxed Warning.

Long-term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver.

Pregnancy Category X

See **CONTRAINDICATIONS** and Boxed Warning.

Estrogens should not be used during pregnancy.

Nursing Mothers

As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS

See **WARNINGS** and Boxed Warning regarding potential adverse effects on the fetus, induction of malignant neoplasms, increased incidence of gallbladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.

The most commonly reported adverse reaction to Estraderm in clinical trials was redness and irritation at the application site. This occurred in about 17% of the women treated and caused approximately 2% to discontinue therapy.

The following additional adverse reactions have been reported with estrogenic therapy, including oral contraceptives:

Genitourinary System: Breakthrough bleeding; spotting; change in menstrual flow; increase in size of uterine fibromyomata; change in cervical erosion and amount of cervical secretion.

Endocrine: Breast tenderness, breast enlargement.

Gastrointestinal: Nausea, vomiting; abdominal cramps, bloating; cholestatic jaundice have been observed with oral estrogen therapy.

Eyes: Steepening of corneal curvature; intolerance to contact lenses.

Central Nervous System: Headache, migraine, dizziness.

Miscellaneous: Change in weight, edema, change in libido.

Do not store above 86°F (30°C).

Do not store unpouched. Apply immediately upon removal from the protective pouch.

CAUTION: Federal law prohibits dispensing without prescription.

C I B A

CIBA Pharmaceutical Company, Division of CIBA-GEIGY Corporation
Summit, New Jersey 07901

© 1986, CIBA.

External vibratory acoustic stimulation near term: Fetal heart rate and heart rate variability responses

Robert Gagnon, M.D., Cora Hunse, R.N., Lesley Carmichael, B.Sc., Fraser Fellows, M.D., and John Patrick, M.D.*

London, Ontario, Canada

Twenty-five pregnant women between 36 and 40 weeks' gestational age were studied to examine effects of a 5-second external vibratory acoustic stimulus on the fetal heart rate and fetal heart rate variability. There was an immediate significant increase in the basal heart rate, which persisted for 1 hour after the stimulus, compared to that of the control subjects. There was also a significant increase in the total time during which accelerations occurred for the first 20 minutes after the stimulus. There were no changes in computer-derived indices of fetal heart rate variability. Maternal blood pressure and heart rate were not altered following vibratory acoustic stimulus. We hypothesize that external vibratory acoustic stimulus causes a change from a state of sleep to a state of wakefulness in near term healthy fetuses. (AM J OBSTET GYNECOL 1987;156:323-7.)

Key words: Nonstress test, external vibratory acoustic stimulation, effects of sound and vibration on fetal heart rate

The close association between human fetal heart rate accelerations and fetal health has been largely documented.[1-3] However, because healthy fetuses may be at rest for as long as 80 minutes, there has been a high false positive rate for nonstress tests performed over 20 or 40 minutes.[3] Use of external vibratory acoustic stimulus has been suggested to increase the reactivity of human fetuses.[4-5] We previously reported that the pattern in fetal heart rate was altered for at least 15 minutes following a 5-second stimulus with an electronic artificial larynx.[6]

The purpose of this study was to define, in a group of healthy fetuses near term, changes in fetal heart rate, fetal heart rate variability, maternal blood pressure, and heart rate after a standardized external vibratory acoustic stimulus.

Patients and methods

Patients. Informed consent was obtained from 25 pregnant women between 36 and 40 weeks' gestational age. Twenty-one women remained in good health, and four subsequently developed mild preeclampsia. Fetal outcome confirmed gestational age and the good health of fetuses studied. The average gestational age at delivery was 39.6 ± 0.2 weeks. The average weight of fetuses was 3373 ± 75 gm (SEM). The average 5-minute Apgar score was 9.3 ± 0.1. No Apgar score was <8 at 5 minutes. Umbilical cord blood gas values were determined in 18 of the fetuses. The average umbilical arterial Po_2 was 16.6 ± 0.9 mm Hg, Pco_2 was 49 ± 2.5 mm Hg, and pH was 7.27 ± 0.02. There were five cesarean section deliveries: one for cephalopelvic disproportion, one for development of acute fetal distress in labor with prolapsed cord (5-minute Apgar score of 8), and three repeat cesarean sections. Twenty-four fetuses were in vertex presentation and one in breech presentation at the time of delivery.

Experimental design. Women received a standardized 800 kcal breakfast or lunch at 8 AM (n = 15) or 12 noon (n = 13). All studies began an hour later and were conducted in a quiet room with patients either sitting upright in bed or resting in a lateral recumbent position. Women were asked not to smoke for at least 4 hours before the studies. Smoking or food intake by patients or observers was not permitted during the study.

All women had been acclimatized by listening to the stimulus in air before the beginning of the studies and were informed that the stimulus might be applied either on their abdomen or their hand. Three women had both control and stimulus studies, and observations were separated by >2 days.

The external vibratory acoustic stimulus used in this

From the Departments of Obstetrics and Gynecology and Physiology, University of Western Ontario, St. Joseph's Hospital Research Institute.

This work was supported by the Physicians' Services Incorporated Foundation. R. G. is supported by a fellowship from the Variety Club of Ontario.

Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.

Reprint requests: Dr. John Patrick, Department of Obstetrics and Gynecology, St. Joseph's Hospital, 268 Grosvenor St., London, Ontario, Canada N6A 4V2.

**Member of the Canadian Medical Research Council Group in Reproductive Biology.*

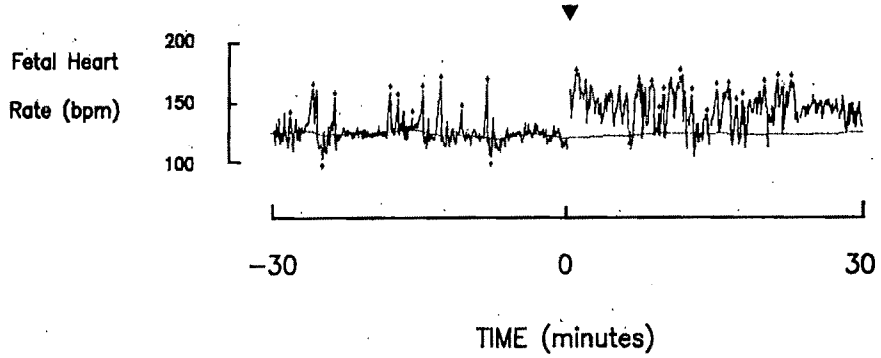


Fig. 1. A typical example of an increase in basal fetal heart rate and FHR accelerations following the application of a 5-second vibratory acoustic stimulus with the electronic artificial larynx.

study was generated with a Model 5C electronic artificial larynx (Western Electric, New York, New York, courtesy of Mr. John Kasper, Corometrics Medical Systems) as previously described.[6] The device produced a form of white noise between 10 and 10,000 Hz as measured in air at 1 cm from the generating source by a real-time spectrum analyzer (Brüel and Kjaer digital frequency analyzer, Type 2131). Maximum sound pressure level was 110 db at 10,000 Hz. The surface of the instrument vibrated at all frequencies between 10 and 20,000 Hz and maximum vibration occurred at 450 Hz (measurements made at 2 cm from the source with a Fotonic sensor, MTI Instruments Division). Women were studied continuously for 2 hours. After the first hour of observation, a 5 second vibratory acoustic stimulus with the electronic artificial larynx was applied on the surface of the maternal abdomen over the fetal head (n = 14) or on the maternal hand for control (n = 14). The choice of whether to stimulate the abdomen or hand of women was made from random number tables, and all women received only one stimulus to either abdomen or hand during individual studies.

Fetal and maternal cardiovascular measurements. Fetal heart rate was recorded with a Hewlett-Packard 8040A (Hewlett-Packard GMBH, Boeblinger, West Germany) external monitor with use of a compact transducer and was analyzed on-line by a microprocessor. The system consisted of a master 512K-memory microprocessor, Sage II (Sage Computer Technology, Reno, Nevada), and dual floppy disc drive, keyboard, and visual display unit as described by Dawes et al.[7] The Hewlett-Packard external monitor was interfaced with the microprocessor by a voltage/frequency converter, which was adjusted to generate a brief fast rising voltage pulse at intervals corresponding to the calculated heart rate. The fetal pulse intervals were examined with use of an artifact detection algorithm[8] and valid pulse intervals were averaged over 3.75 seconds. During analysis a baseline was fitted to the heart rate record as previously described,[9] and accelerations of >10 bpm for ≥15 seconds above the baseline were recognized automatically.

For each 10-minute interval the basal fetal heart rate (beats per minute), the root mean square (milliseconds) and the mean minute range (milliseconds) were calculated. The root mean square and the mean minute range quantify long-term fetal heart rate (FHR) variability.[7] To identify maternal cardiovascular effects, the maternal heart rate, systolic blood pressure, diastolic blood pressure, and mean arterial blood pressure were automatically recorded every 5-minute with a Dinamap (Model 845XT, Critikon, Inc., Tampa, Florida).

Fetal breathing movements and gross fetal body movements were measured with a real-time ADR ultrasound scanner (Model 2130, Advanced Diagnostic Research Corp., Tempe, Arizona) and were reported elsewhere.[10]

Data analysis. The basal fetal heart rate, number of accelerations of >10 bpm for ≥15 seconds above the baseline, duration and amplitude of individual accelerations, the total time spent in acceleration, the root mean square, and the mean minute range were determined for successive 10-minute intervals. Maternal heart rate and blood pressure were analyzed as mean ± SEM at 5-minute intervals. All other results are presented as means ± SEM for each 10-minute interval before and after either vibratory acoustic stimulus on the maternal abdomen (stimulus) or on the maternal hand (control) for the 2-hour observation periods. Portions of records that could not be analyzed because of signal loss of >30% were not included. These portions made up 2.1% of total study time.

Statistical significance between groups before and after stimulus or control was determined by use of a mixed nested analysis of variance (ANOVA) comparing overall means and each of the 10-minute intervals[11] with an HP 9845B microprocessor (Hewlett-Packard, Computer Division, Fort Collins, Colorado, USA).

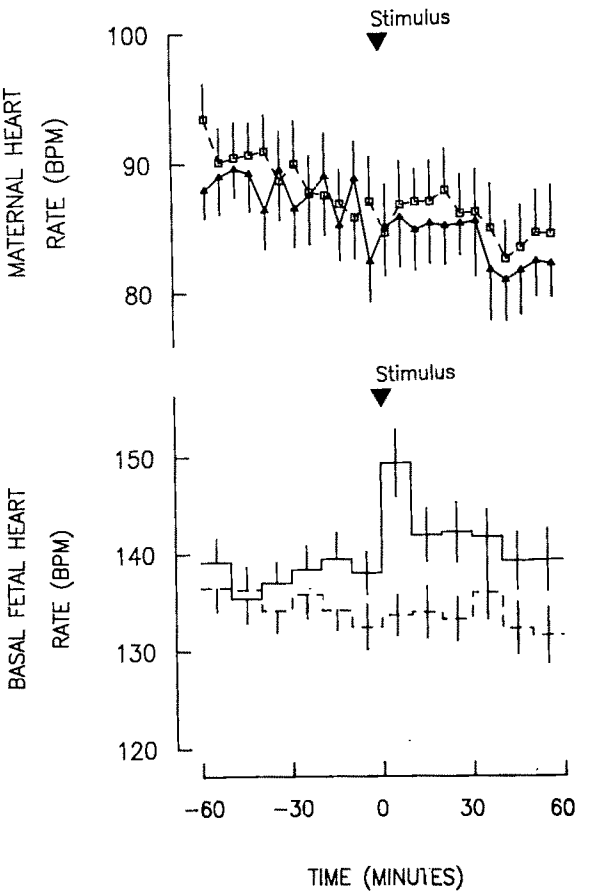


Fig. 2. The mean maternal heart rate was plotted in 5-minute intervals before and after control *(dashed line)* or stimulus *(solid line)*. The mean basal fetal heart rate was also plotted in 10-minute intervals before and after control *(dashed line)* or stimulus *(solid line)*.

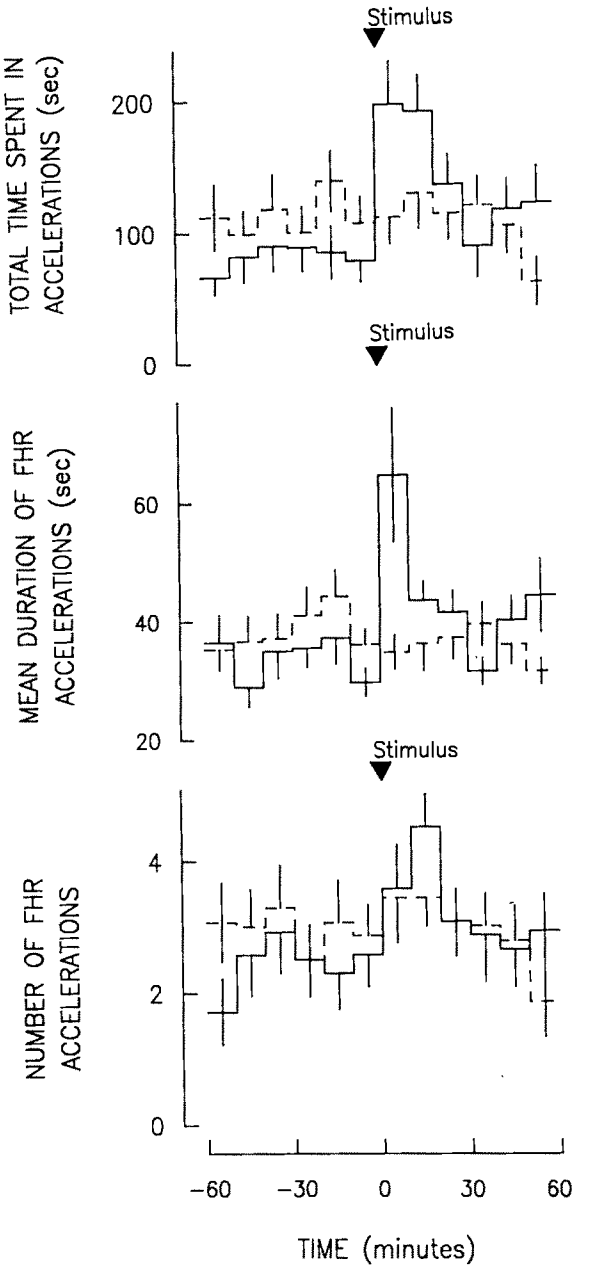


Fig. 3. The mean time spent in accelerations was plotted in 10-minute intervals before and after control *(dashed line)* or stimulus *(solid line)*. The mean duration of FHR accelerations and the mean number of FHR accelerations were also plotted on the same time scale.

Results

Fetal heart rate. A typical example of the FHR response to vibratory acoustic stimulus is seen in Fig. 1. Analysis of data demonstrated the mean basal FHR was 135 ± 2.1 bpm before control, which was not different from 138.4 ± 2.2 bpm before stimulus. There was an increase in the mean basal FHR during the hour after the stimulus of up to 142.1 ± 2.7 bpm, which was significantly greater than that after the control (Fig. 2) (ANOVA, $p = 0.017$). The maximum increase in basal FHR (149.2 ± 3.0 bpm) occurred during the first 10 minutes after the stimulus and remained above the control for the hour after the stimulus (ANOVA, $p = 0.0004$). During the control study the basal FHR decreased to 133.4 ± 2.2 bpm, which was similar to the decrease in maternal heart rate (Fig. 2).

Characteristics of FHR accelerations. Fig. 3 summarizes the characteristics of FHR acceleration responses per 10-minute interval before and after either control or stimulus. The total time per 10-minute interval during which accelerations occurred was 81 ± 10 seconds before the stimulus, and it increased to 142 ± 19 sec after the stimulus. ANOVA revealed there was a significant increase in the total time spent in accelerations during the first 20 minutes after the stimulus compared to control (ANOVA, $p = 0.0014$).

The mean duration of FHR accelerations (Fig. 3) was 33.0 ± 1.2 seconds during the hour before stimulus and 44.1 ± 2.2 seconds after stimulus. This was significantly longer than after control (ANOVA, p =

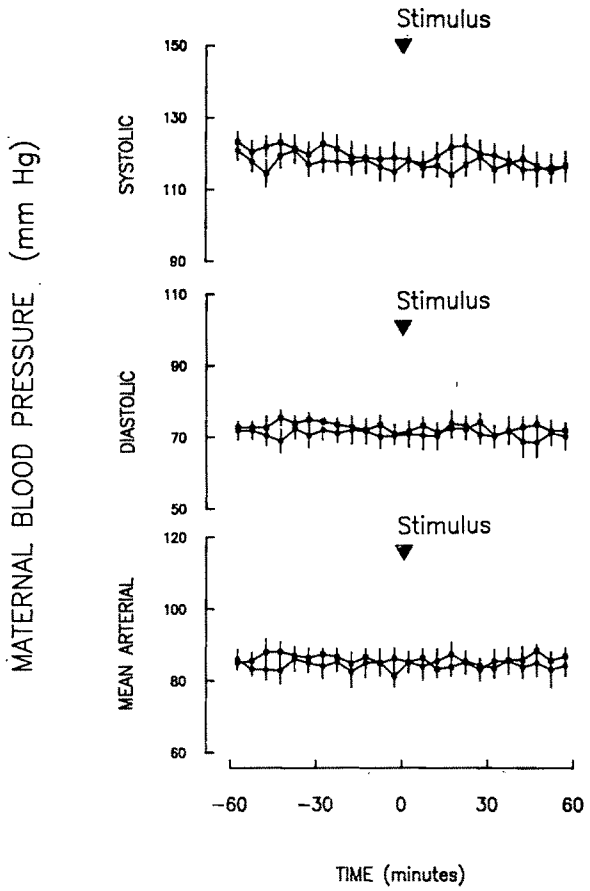


Fig. 4. The maternal systolic, diastolic, and mean arterial blood pressure was plotted in 5-minute intervals before and after control *(dashed line)* or stimulus *(solid line)*.

0.0031). ANOVA demonstrated a significant increase in the mean duration of FHR accelerations (64.5 ± 11.3 sec) during the first 10 minutes after the stimulus (ANOVA, $p = 0.0128$).

There were 2.5 ± 0.3 FHR accelerations per 10-minute interval before stimulus (Fig. 3), and ANOVA demonstrated a significant increase in the number of FHR accelerations between 10 and 20 minutes after the stimulus (ANOVA, $p = 0.0271$). All fetuses that received stimuli exhibited accelerations in the FHR between 10 and 20 minutes after vibratory acoustic stimulation.

The mean amplitude of FHR accelerations was 17.5 ± 2 bpm before stimulus and was not different in the 2 hours after either stimulus or control.

Fetal heart rate variability. The mean minute range per 10-minute interval was 44.6 ± 4.1 msec (range, 25 to 84 msec) and did not change after stimulus. The median root mean square value per 10-minute interval was 8.6 msec (range, 4.6 to 17.3 msec) before stimulus and did not change following vibratory acoustic stimulation.

Maternal blood pressure and heart rate. Fig. 4 demonstrates that systolic, diastolic, and mean arterial blood pressures were not changed by external vibratory acoustic stimulation. Mean maternal heart rate tended to decrease slightly during the experiment but was not significantly different before or after control or stimulus (Fig. 2).

Comment

This study made use of on-line analysis by a microprocessor that was able to quantify more accurately the different components of fetal heart rate. The results indicated that the pattern in the fetal heart rate after application of a vibratory acoustic stimulus on the surface of the maternal abdomen was altered for up to 1 hour after the 5-second stimulus. There was an increase of 11 bpm in the basal heart rate which began immediately after the stimulus and remained elevated above control for one hour after the stimulus. There was also a significant increase in the total time during which accelerations occurred in the first 20 minutes after stimulus which was due to (1) increase in the duration of FHR accelerations in the first 10-minute interval and (2) an increase in the number of FHR accelerations during the second 10-minute interval. The root mean square and mean minute range, which are measurements of long-term FHR variability, did not change. Similarly, maternal blood pressure and heart rate were not changed by the vibratory acoustic stimulus.

Prechtl et al.[12] studied term newborn infants and defined five different behavioral states that characterized sleep and wakefulness.[12] Dehaan et al.,[13] reported that in term neonates both heart rate and heart rate variability were increased during active sleep compared to quiet sleep. They also reported that during neonatal wakefulness the heart rate was higher but the variability was the same as during quiet sleep.[13]

The five states of sleep and wakefulness originally described in neonates are believed to take their origin in late fetal life in humans. Nijhuis et al.[14] with use of real-time ultrasound observed that fetuses of 36 weeks' gestation had developed states that were, in their organization, fully comparable to the states originally described by Prechtl et al. in neonates. Nijhuis et al.[14] described four different states, and one of these was characterized by a fetal heart rate with large and prolonged accelerations, often fused into sustained tachycardia. They believed these were periods of wakefulness. Patrick et al.[15] have defined the normal pattern of FHR accelerations was 40 seconds with a significant increase of up to 57 seconds between 11 PM and 2 AM coincidentally with an increase in the incidence of gross ncrease of up to 57 seconds between 11 PM and 2 AM coincidentally with an increase in the incidence of gross fetal body movements at this time period. This sort of pattern in healthy neonates is observed only during times of wakefulness and they speculated that human

fetuses at term may have prolonged periods of wakefulness in the late evening.

The FHR response to vibratory acoustic stimulation defined in this study was characterized by an increase in basal FHR and duration and number of FHR accelerations in the absence of changes in FHR variability. These observations would be compatible with the hypothesis that fetuses have been changed from a state of sleep to a state of wakefulness as a result of vibratory acoustic stimulation.

Schmidt et al.[16] reported that external controlled sound stimulation at 2000 Hz (120 db) of 5-second duration with a loudspeaker separated from the maternal skin by a ring of foam rubber failed to elicit any change in FHR or fetal body movement patterns.[16] Therefore the FHR response to vibratory acoustic stimulus in this study may be a result of stimulation by the vibrational component rather than the acoustic component of the electronic artificial larynx.

It remains to be determined whether a similar FHR response to stimulus with the electronic artificial larynx will occur in fetuses earlier in pregnancy. Data are also required regarding negative and positive predictive value for fetal outcome before suggesting any clinical application of the electronic artificial larynx in the evaluation of fetal health.

We wish to thank Professor G. S. Dawes and Mr. Isaac Rapoport for their kind assistance.

REFERENCES

1. Pratt D, Diamond F, Yen H, Bieniarz J, Burd L. Fetal stress and nonstress tests: an analysis and comparison of their ability to identify fetal outcome. Obstet Gynecol 1979;54:419.
2. Phelan JP. The nonstress test: a review of 3000 tests. Am J Obstet Gynecol 1981;139:7.
3. Brown R, Patrick J. The nonstress test: how long is enough? Am J Obstet Gynecol 1981;141:646.
4. Ohel G, Birkenfeld A, Rabinowitz R, Sadovsky E. Fetal response to vibratory acoustic stimulation in periods of low heart rate reactivity and low activity. Am J Obstet Gynecol 1986;154:619.
5. Davey DA, Dommisse J, Macnab M, Dacre D. The value of an auditory stimulatory test in antenatal fetal cardiotocography. Eur J Obstet Gynecol Reprod Biol 1984;18:273.
6. Gagnon R, Patrick J, Foreman J, West R. Stimulation of human fetuses with sound and vibration. Am J Obstet Gynecol 1986;155:848-51.
7. Dawes GS, Redman CWG, Smith JH. Improvements in the registration and analysis of fetal heart rate records at the bedside. Br J Obstet Gynaecol 1985;92:317.
8. Dalton KJ, Dawes GS, Patrick JE. Diurnal, respiratory, and other rhythms of fetal heart rate in lambs. Am J Obstet Gynecol 1977;127:414.
9. Dawes GS, Houghton CRS, Redman CWG. Baseline in human fetal heart-rate records. Br J Obstet Gynaecol 1982;89:270.
10. Gagnon R, Hunse C, Carmichael L, Fellows F, Patrick J. Effects of vibratory acoustic stimulation on human fetal breathing and gross fetal body movements near term. Am J Obstet Gynecol 1986;155:1127-30.
11. Hicks CR. Fundamental concepts in the design of experiments. 2nd ed. New York: Holt, Rinehart & Winston, 1973:100.
12. Prechtl HFR, Akiyama Y, Zinkin P, Kerr Grant D. Polygraphic studies of the fullterm newborn infants. I. Technical aspects and qualitative analysis. In: Bax MCO, Mackeith RC, eds. London: Heinemann, 1968:1.
13. Dehaan R, Patrick J, Chess GF, Jaco NT. Definition of sleep state in the newborn infant by heart rate analysis. Am J Obstet Gynecol 1977;127:753.
14. Nijhuis JG, Prechtl HFR, Martin CB Jr, Bots RSGM. Are there behavioural states in the human fetus? Early Hum Dev 1982;6:177.
15. Patrick J, Carmichael L, Chess L, Staples C. Accelerations of the human fetal heart rate at 38 to 40 weeks' gestational age. Am J Obstet Gynecol 1984;148:35.
16. Schmidt W, Boos R, Gnirs LA, Schulze S. Fetal behavioural states and controlled sound stimulation. Early Hum Dev 1985;12:145.

Psychological adaptation to pregnancy in childless women more than 35 years of age

G. Erlick Robinson, M.D., David M. Garner, Ph.D., Douglas J. Gare, M.D., and Belinda Crawford, B.Sc.

Toronto, Ontario, Canada

Forty-five pregnant childless women with a mean age of 36.6 years (±1.7) were compared with 42 nulliparous pregnant women with a mean age of 26.7 years (±2.8). Initial interviews determined the basic demographic data, the reasons for delaying pregnancy, attitudes to pregnancy, and plans concerning careers. Psychological tests were given to measure anxiety, depression, anger, marital intimacy, sense of autonomy, and role expectations. These psychological tests were applied to all patients at 12 to 16 weeks' gestation and again at 28 to 32 weeks' gestation. The demographic information from the two groups was remarkably similar. Although their ages differed by 10 years, their career goals, their family incomes, reasons for delaying pregnancy, and reasons for now getting pregnant revealed minimal differences. The Maffer Feminine Identity Scale revealed that the younger women held more traditional attitudes toward a woman's roles than the older group. The Sociotrophy-Autonomy Scale indicated that the older women were more independent than the younger pregnant patients. The Symptom Checklist indicated that the older group were less troubled by pregnancy than the control group, and although their level of distress increased as the pregnancy progressed, they remained better adjusted as they entered the last trimester of their pregnancies. (AM J OBSTET GYNECOL 1987;156:328-33.)

Key words: Elderly primiparas, pregnancy, psychological adjustment, emotional adjustment

In recent years, reliable methods of birth control and the changing roles of women have led to an increasing number of women postponing childbirth. In 1980, more than 13,000 women in their 30s gave birth to their first child; this represents a 94% increase since 1975. Previous research has focused on the psychological and social changes occurring in young primiparous women during this transition from childlessness to being a parent. Considering the trend toward postponing childbirth, it is surprising that there has been relatively little research into the psychological impact of giving birth at midlife. Baumann reported to the International Association of Psychosomatic Obstetrics and Gynecology that he had examined reasons for delaying pregnancy in a group of women of different ages and concluded that the older women were unconventional in the sense that a high percentage lived with younger partners. The main reasons reported for delayed childbearing were late acquaintance of the right partner and the wish to be free of responsibility. Unfortunately these women were interviewed only at the time of delivery, which may have influenced their recall of certain events.

A more detailed understanding of the pattern of adjustment in older primiparous women may lead to more specific recommendations to this group and, if they experience more psychosocial difficulties, it may result in more appropriate support during the pregnancy and the postpartum period. The aim of the present study was to examine with standardized psychometric measures the patterns of adjustment to pregnancy in a group of childless women who were 35 or older and compare them with a group of younger women who had never given birth.

Patients and methods

The present study was based on interview and psychometric data obtained in a group of 45 childless women over the age of 35 and a group of 42 women 30 years of age and under who also had never given birth. Both groups were interviewed initially between 12 and 16 weeks' gestation. The interview consisted of 30 questions to obtain demographic data as well as information concerning reasons for the timing of the current pregnancy, attitudes toward pregnancy, and plans concerning child care and work. In all cases permission to interview the women was obtained from the appropriate physician and written informed consent was acquired from the prospective mother.

From the Departments of Psychiatry and Obstetrics and Gynaecology, Toronto General Hospital, University of Toronto.

Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986, and in part at the American Society of Psychosomatic Obstetrics and Gynecology, Sarasota, 1984.

Reprint requests: Dr. G. Erlick Robinson, Department of Psychiatry, 8-231 Eaton Bldg. North, Toronto General Hospital, Toronto, Ontario, Canada M5G 2C4.

Table I. Circumstances surrounding pregnancy for older and younger childless women

	Older primiparous women (n = 42)		*Younger primiparous women (n = 42)*			
	No.	*%*	*No.*	*%*	χ^2	$p \leq$
Reason for pregnancy						
Both emotionally ready	22	48.9	34	81.0	8.34	≤0.004
Found suitable mate	19	42.2	5	11.9	8.54	≤0.004
Suitable point in career	10	22.2	2	4.8	4.20	≤0.04
Financially feasible	9	20.0	12	28.6	0.47	NS
Mother emotionally ready	10	22.2	4	9.5	1.73	NS
Infertility solved	7	15.6	1	2.4	3.08	NS
Family pressure	0	0	1	2.4	0.001	NS
Marriage stable now	4	8.9	1	2.4	0.71	NS
Delay of pregnancy for						
No suitable partner	25	55.6	9	21.4	9.24	≤0.002
Both not ready	10	22.2	20	47.6	5.13	≤0.02
Infertility	7	15.6	1	2.4	3.08	NS
Career	8	17.8	4	9.5	0.64	NS
Financial reasons	5	11.1	16	38.1	7.23	≤0.007
Pregnancy planned	35	77.8	34	81.0	0.01	NS
Very satisfied with being pregnant	39	86.7	36	85.7	0.00	NS

Psychometric testing was then carried out on two occasions: The first session was before viability (at 12 to 16 weeks' gestation) and the second was between 28 and 32 weeks' gestation. The final groups did not include three women who refused to participate after the initial interview, 15 women who initially agreed to participate but did not return the first package of psychometric measures, and one woman who dropped out after initial testing. Of the women who completed the initial testing, one had a spontaneous abortion, one underwent abortion for genetic reasons, and another was excluded from the data analysis because her child was born with a serious birth defect.

At the initial contact the women were given a package consisting of the following questionnaires with the instructions to return them within 1 week: (1) the Symptom Checklist, which is a 90-item self-report measure designed to assess mood states including anxiety, depression, somaticism, hostility, phobia, obsessionality, paranoia, and psychoticism; (2) the Maffer Feminine Identity Scale, which is a 24-item scale that measures traditional and nontraditional sex role orientation; (3) the Social Intimacy Scale, which is a 20-item self-report measure of perceived intimacy with a significant other; (4) the Sociotropy-Autonomy Scale, which is a self-report personality measure that provides scores for social dependence (the degree to which social relationships are perceived as important) and autonomy (the degree to which independent pursuits are perceived as important).

On the second testing occasion, the women were sent a package of self-report measures in the mail with instructions to return them within 1 week. The following measures were completed: (1) the Cranley Maternal-Fetal Attachment Scale, which is a 24-item measure with five subscales (role taking, differentiation of self from the fetus, interaction with the fetus, attributing characteristics to the fetus, and giving of self) designed to assess the degree of maternal bonding toward the fetus; (2) the Symptom Checklist; and (3) the Social Intimacy Scale described above.

Data analysis. Group differences on categorical variables were analyzed with the use of the χ^2 statistic and Fischer's correction, where appropriate. Continuous variables were exposed to a two-way analysis of variance (group × testing occasion) except when measures were administered only once, and then variables were compared with the use of Student's t test (two-tailed).

Results

As expected, there were age differences between groups (older, mean = 36.6 ± 1.7; younger, mean = 26.7 ± 2.8; $t = 19.54$, $p < 0.0001$); however, there were no differences between groups in the number of years married (older, mean = 4.1 ± 4.1; younger, mean = 3.4 ± 2.3). Similar proportions of the two groups of women had careers (older, 50.0%; younger, 28.2%; $\chi^2 = 3.24$, NS), incomes greater than $25,000 (older, 55.6%; younger, 43.6%; $\chi^2 = 0.77$, NS), and partners with incomes greater than $25,000 (older, 78.6%; younger, 66.7%; $\chi^2 = 0.96$, NS). Significantly more of the older women (62.2%) than the younger group (38.1%) had a university education ($\chi^2 = 4.14$, $p < 0.05$). The older group had significantly more dif-

Table II. Scores on the Maffer Feminine Identity Scale and the Sociotropy-Autonomy Scale for older and younger childless women

	Older women (n = 45) (mean ± SD)	*Younger women (n = 41) (mean ± SD)*	*t Value*	*p≤*
Maffer Feminine Identity score*	58.8 ± 10.1	66.0 ± 11.4	3.1	≤0.003
Sociotropy (total)	51.2 ± 15.5	61.7 ± 17.0	3.0	≤0.004
Concern re disapproval	13.0 ± 6.0	15.0 ± 6.6	1.4	NS
Attachment	25.8 ± 7.4	32.4 ± 6.7	4.3	≤0.00
Pleasing others	12.2 ± 4.7	13.6 ± 5.5	1.3	NS
Autonomy (total)	70.0 ± 13.9	63.9 ± 11.3	−2.5	≤0.02
Autonomous achievement	32.3 ± 5.9	29.6 ± 5.3	−2.2	≤0.03
Freedom from others' control	25.5 ± 6.5	22.8 ± 6.3	−2.0	≤0.05
Preference for solitude	13.4 ± 3.6	11.5 ± 2.7	−2.7	≤0.003

*High score indicates more traditional values.

ficulty conceiving with 1.1 (±2.4) years' delay versus 0.3 (±0.7) year for the younger group (t = −2.18).

With regard to obstetric performance, the duration of labor, Apgar scores at 1 and 5 minutes, weights of newborn infants, and neonatal course of the infants, there were no statistically significant group differences. Three significant perinatal problems occurred in the younger or control group, and these included one case of bilateral talipes equinovarus, one case of pneumothorax with pneumomediastinum, and a case of duodenal atresia. Similarly there were three significant neonatal problems in the babies delivered of mothers over age 35. These included a ventricular septal defect, which was repaired, a cleft palate, and one case of osteogenesis imperfecta.

As indicated in Table I, various reasons were given for delaying pregnancy in the younger and older groups. In the older group, the majority claimed that they had not had a suitable partner, less than a quarter stated that both partners were not yet ready to have children, and just over 15% identified infertility as the reason for delaying pregnancy. In the younger group, the most common reason for delaying concerned both partners not being ready for children followed by financial constraints. The majority of both the older and the younger women reported that they were satisfied with the pregnancy and that the event had been planned. The most common reason for becoming pregnant in both groups was emotional readiness followed by finding a suitable mate for the older group and financial feasibility for the younger group.

The history of previous pregnancies indicates that 20 elderly primiparous women and 16 younger primiparous women had previously had at least one abortion. In these subgroups, elective abortion was the most common (45.0% in the older subgroup and 56.3% in the younger subgroup), but spontaneous abortions were also common (40.0% of the older group and 37.5% of the younger group). Of the 11 older primiparous women who had had multiple abortions, 36.4% had been elective and 54.5% spontaneous. Only two of the younger women had had more than one elective abortion.

The results of the two groups of women on both the Maffer Identity Scale and the Sociotropy-Autonomy Scale were compared with *t* tests, with a Bonferroni-type adjustment used to control for Type I error due to the multiple comparisons. The younger women had significantly higher scores than the older women on the Maffer Feminine Identity Scale, indicating that the younger women had more traditional views. The younger women were significantly more sociotropic than the older women, particularly with respect to attachment to others or concern about separation, while the older women were more autonomous than the younger women, especially on the preference for solitude factor. The Cranley Maternal-Fetal Attachment Scale was administered only at 28 to 32 weeks' gestation and there were no significant group differences on four of the five subscales; the younger group had higher scores on the Differentiation Subscale (older, mean = 15.9 ± 2.7; younger, mean = ±2.1; t = 2.32, p = 0.03) (Table II).

The Symptom Checklist and the Social Intimacy Scale were administered at 12 to 16 weeks' gestation and at 28 to 32 weeks' gestation. These data were subjected to analysis of variance with one between-subjects factor (age) and one within-subjects factor (stage of gestation). There were no significant group or time differences on the Social Intimacy Scale. As indicated in Table III, there were group, time, or interaction effects on most of the Symptom Checklist subscales. There were significant time of gestation effects for somatizing, obsessive-compulsive, depression, anxiety, and psychoticism subscales with both groups of women reporting more symptoms at the second testing than at

Table III. Score on the SCL 90 for older and younger childless women

Subscale	Older women (n = 35) (mean ± SD)	Younger women (n = 33) (mean ± SD)	Probability of effect		
			Group	Time	Group × time interaction
Somatization					
First time	4.0 ± 4.1	7.5 ± 4.9	0.005	0.001	NS
Second time	6.7 ± 4.7	9.0 ± 5.8			
Obsessive-compulsive					
First time	3.5 ± 3.7	5.2 ± 3.1	NS	0.002	NS
Second time	5.4 ± 5.2	6.2 ± 4.7			
Intimacy					
First time	2.4 ± 2.7	4.7 ± 3.2	0.008	NS	NS
Second time	2.6 ± 3.1	4.5 ± 4.3			
Depression					
First time	4.5 ± 4.5	9.3 ± 6.5	0.01	0.03	0.02
Second time	7.4 ± 6.4	9.2 ± 5.9			
Anxiety					
First time	2.0 ± 2.2	3.9 ± 3.3	0.04	0.05	NS
Second time	3.2 ± 3.4	4.3 ± 4.6			
Hostility					
First time	1.8 ± 2.2	3.5 ± 3.2	0.05	NS	NS
Second time	2.3 ± 3.3	3.1 ± 3.3			
Phobia					
First time	0.49 ± 1.1	1.4 ± 1.4	0.006	NS	NS
Second time	0.83 ± 1.2	2.1 ± 3.3			
Paranoia					
First time	1.7 ± 2.6	2.2 ± 2.6	NS	NS	NS
Second time	1.6 ± 2.6	2.6 ± 3.0			
Psychoticism					
First time	0.97 ± 1.8	1.4 ± 2.6	NS	NS	NS
Second time	1.5 ± 3.3	2.2 ± 3.6			

the first testing. The younger women displayed significantly more distress than the older women on somatizing, interpersonal sensitivity, depression, anxiety, hostility, and phobia scales. There were no significant group-by-time interactions except on the depression subscale, indicating that the older women increased in depression over time while the younger women did not; however, at the second testing the older women were still less depressed than the younger women. The total scores on the Symptom Checklist are summarized in Fig. 1, which indicates that both groups became significantly more distressed over time and that the younger women overall report more distress than the older women.

Comment

A woman's psychological adaptation may influence the course of pregnancy, the labor and delivery, and the postpartum period. Emotional disorders in pregnancy have been related to low self-esteem, previous psychiatric history, being unmarried, marital problems, and environmental stresses. Negative attitudes during pregnancy have been shown to be related to intense pain and higher levels of medication during labor. These findings are supported by McDonald,[1] who found a positive correlation between anxiety and labor length.

Blum[2] retrospectively compared the pregnancies of 55 elderly primiparous women with those of two groups of primiparous women aged 20 to 29 and 30 to 34 and found that the older women were more careful during pregnancy and consulted their physicians more often. Zajicek[3] found that the younger women in his study were more likely to experience psychiatric problems both before and after birth, but 47% of his younger group were teenagers when they became pregnant. In contrast, Hayworth et al.[4] found that younger women and women who perceived themselves as less in control of their lives were more likely to be depressed postnatally.

Previous research provides few insights into the degree to which observations derived from younger primiparous women may be generalized to women who have their first child somewhat later in life. Older women who become parents for the first time may differ from younger women in many ways. They may be more financially stable and independent. They may have already established careers that have added to their sense of self-esteem, maturity, and role identification. Moreover, careers may be greatly influenced by the presence of a child. Having had relative freedom or the presence of only a dyadic relationship for many years, the woman may have difficulty adjusting to the increased ties and responsibilities of a child. The hus-

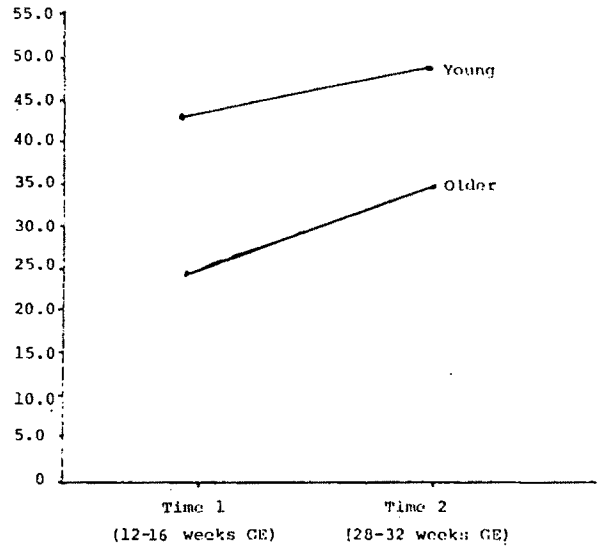


Fig. 1. Total score on the Symptom Checklist for older and younger childless women. A two-way analysis of variance demonstrated a significant group effect ($p \leq 0.01$) and a significant time effect ($p \leq 0.007$).

band may have had a longer period of accepting the present sex role of his wife and may not expect her to suddenly shift to a more traditional pattern.

The results of the current prospective study indicate that the older group of pregnant women make a relatively positive adjustment to pregnancy. At 3 and 7 months before delivery they demonstrate less distress than the younger women as measured by the Symptom Checklist. There are certain limitations that must be noted concerning group selection since it was not possible to randomly allocate women to groups that would have a first child in their 20s versus those who would wait until they were 35 or older. Thus it is not possible to conclude that women who wait longer to have children will fare better during pregnancy. It may merely indicate that women who delay pregnancy are a different and perhaps more robust group than those who choose to have their children earlier in life.

Moreover, the degree to which the two groups are representative of the general population is unknown. Both of the studied groups tend to be from the middle or upper-middle class socioeconomic strata. The younger women were obtained from the practices of physicians working in a large, university-affiliated metropolitan hospital. Although some of the older women were drawn from practices of the physicians at the hospital, many of the women were referred to the amniocentesis clinic of the hospital; the degree to which selection based on willingness to undergo amniocentesis affected the findings is uknown. It is quite plausible that women who delay pregnancy do tend to come from higher socioeconomic groups. Nevertheless, the fact that the groups were well matched in terms of most of the demographic factors allows us to compare the effects of differences in age without the influence of factors that have previously been shown to be associated with distress during pregnancy such as low social class and economic problems.

In the current study the reason most often reported by the older women as reponsible for delaying pregnancy was lack of a suitable partner. For some of them, this meant not having previously been married, whereas for others it meant having had an unsatisfactory earlier relationship. It is interesting to note the high degree of planned pregnancies in our two groups (77.8% and 81.0%). Other authors have found a lower percentage of planning in their samples: 41%,[5] 33%,[6] and 52%.[7] The relationship between planning a pregnancy and successful adaptation to pregnancy is not clear. Shereshevsky and Yarrow[8] found that planning was not related to adaptation to pregnancy, and Entwisle and Doering[9] found it not related to the woman's attitude toward being pregnant. On the other hand, Zajicek[3] found that women who did not plan their pregnancies were less likely to react positively throughout the pregnancy. Lederman[1c] also found that women who had planned or wanted their pregnancy were well adjusted.

A number of authors have commented on the change in women's roles associated with having a child. Gordon et al.[11] found that abnormal emotional reactions to pregnancy were related to a maternal role conflict, which indicated that mothers were prepared for social, educational, and economic advancement outside the home but not for the maternal role. Lederman[10] described conflict being acute if the need to be a mother and the need to identify oneself as a career woman were both felt intensely. The findings of the current study indicated that the younger group held more traditional attitudes than the older women, which may simply corroborate the fact that the older group was less traditional by virtue of their delaying of pregnancy. This explanation is weakened by the fact that there were no group differences in terms of numbers of years married, career, or income. Nevertheless, more of the older group had attended a university. These results may be confounded by an overrepresentation of Catholic women in our younger group and these women may have more traditional values. At the same time it may also be that there is a return to more traditional attitudes in many younger women as they see the stress that other women are going through in terms of career and home conflicts. It will be important to determine whether these role attitudes change after the birth of the baby. Consistent with the findings concerning role behavior, the findings from the current study indicated that the older women were more autonomous than the

younger women. Other authors have described the importance of autonomy in older women with careers. It may be that older women as a whole are more autonomous than younger women or that the older women in the current study were more autonomous and therefore chose to become pregnant later.

Many authors have written about the importance of the relationship with the husband in terms of affecting both the course of pregnancy and the postpartum adjustment[8] and even the duration of labor and the need for sedation.[10] Dyer[12] and LeMasters[13] focused on the impact of the coming of a first child on the marital relationship. Both felt this generated crises in the marriage forcing each couple to reorganize many of their roles and relationships. The measure of social intimacy used in the current study failed to reveal significant effects for the women over time. It will be interesting to note if marked changes occur postnatally.

The findings of the current study are consistent with those of other authors who have found increasing psychological tension during the last trimester of pregnancy as women start to face the imminence of labor and birth.[8] The younger group in the current study showed elevated levels on the depression scale at initial testing with only a slight increase on the second testing occasion, which may indicate a ceiling factor of their level of depression rather than an actual differential change in depression between the two groups. However, it cannot be assumed that the younger group are more distressed than nonpregnant women of a similar age. It has been concluded from some studies that women of this age generally have a higher degree of symptom levels than older women. For example, in studies of younger women, Weissman and Klerman[14, 15] have found high levels of depression and Griffith[16] has found increased stress levels, which she hypothesized are pressures associated with heightened expectations and role conflicts in the absence of realistic role models in contemporary society. Younger women may also suffer from more unrealistic optimism and high expectations whereas older women either have achieved valued expectations or place less emphasis on attaining them. It may be that the results of the current study reflect simply the effects of age irrespective of the effect of the pregnancy. However, it is important to note that older childless women do not appear to be unduly distressed during pregnancy despite a perhaps more major change related to this pivotal event in their lives. Not withstanding the greater danger of congenital defects, these women are less symptomatic in the early months of pregnancy. Neither is their continued better adjustment merely the result of the reassurance obtained by having amniocentesis as their depression level still rises over the course of pregnancy.

The postpartum follow-up of these women will elucidate factors during the prenatal period that help to predict outcome. Such predictive factors would then allow preventative measures to be designed to help women who may be vulnerable to problems in the postpartum period.

The Canadian Psychiatric Research Foundation provided support for the pilot project, and The Hospital For Sick Children Foundation and Bell Canada are providing ongoing support.

REFERENCES

1. McDonald RL. The role of emotional factors in obstetric complications: a review. Psychosom Med 1968;30:222-34.
2. Blum M. Is the elderly primipara really at high risk? J Perinat Med 1979;7:108-12.
3. Zajicek E. Psychiatric problems during pregnancy. In: Wolfkind S. Zajicek E, eds. Pregnancy: a psychological and social study. New York: Grune & Stratton, 1981.
4. Hayworth J, Little BC, Carter SB, et al. A predictive study of postpartum depression: some predisposing characteristics. Br J Med Psychol 1980;53:161-7.
5. Breen D. The birth of a first child. London: Tavistock Publications, 1975.
6. Nash FW. Maternal education and attitudes—100 first maternities. Health Soc Serv J 1973;83:2363-4.
7. Cartwright A. How many children? London: Routledge and Kegan Paul, 1976.
8. Shereshevsky PM, Yarrow LJ. Psychological aspects of a first pregnancy and early postnatal adaptation. New York: Raven Press, 1974.
9. Entwisle DR, Doering SG. The first birth. Baltimore: The Johns Hopkins University Press, 1981.
10. Lederman RP. Psychosocial adaptation in pregnancy. Prentice-Hall Inc., New Jersey, 1984.
11. Gordon RE, Kapostins EE, Gordon K. Factors on postpartum emotional adjustment. Obstet Gynecol 1960;25: 158-66.
12. Dyer E. Parenthood as crisis: a re-study. Marriage Fam Liv 1963;25:196-201.
13. LeMasters EE. Parenthood as crisis. In: Susman R, ed. Handbook on the family. Glencoe, Illinois: Free Press, 1964.
14. Weissman MM. The epidemiology of suicide attempts. Arch Gen Psychiatry 1974;30:737-46.
15. Weissman MM, Klerman GL. Sex differences in the epidemiology of depression. Arch Gen Psychiatry 1977;34: 98-111.
16. Griffith JW. Women's stressors according to age groups. Issues Health Care Women 1983;6:311-26.

A complete list of references will be provided on request to the authors.

Shoulder dystocia: Predictors and outcome

A five-year review

Susan J. Gross, M.D., Jerry Shime, M.D., and Dan Farine, M.D.
Toronto, Ontario, Canada

Shoulder dystocia is an uncommon complication of delivery with a high morbidity rate. Ninety-one cases were coded for shoulder dystocia at the Toronto General Hospital from 1980 through 1985. True shoulder dystocia was found in 24 cases, an incidence of 0.23%. There was no significant difference in average weight and percentage of macrosomia between cases of true shoulder dystocia and those merely coded as such. True shoulder dystocia was associated with a neonatal morbidity rate of 42%, consisting of a respiratory arrest and neurological and orthopedic damage. Fundal pressure, in the absence of other maneuvers, resulted in a 77% complication rate and was strongly associated with orthopedic and neurologic damage. Delivery of the posterior shoulder and the corkscrew maneuver were associated with good fetal outcome. (Am J Obstet Gynecol 1987;156:334-6.)

Key words: Shoulder dystocia, complications, obstetrics, delivery maneuvers

Although shoulder dystocia is an uncommon complication of delivery with a suspected incidence of 0.15% to 0.3%,[1] it is associated with a relatively high morbidity rate of 16% to 48%[2, 3] and potentially devastating outcome.

The risk factors for shoulder dystocia are often seen with macrosomia (birth weight of >4000 gm) and include multiparity, postdatism, previous macrosomic infant, and obesity. Diabetes carries a twofold risk of shoulder dystocia that is due to a combination of macrosomia and altered body configuration.[4, 5] Prolonged second stage of labor and midforceps delivery (alone and in combination),[3] as well as protraction and arrest disorders,[6] have also been identified as risk factors for shoulder dystocia.

True shoulder dystocia refers to deliveries requiring maneuvers to deliver the shoulders in addition to downward traction and episiotomy.[1] The following study documents our experience with shoulder dystocia during a 5-year period. Deliveries complicated by true shoulder dystocia were compared to deliveries that were merely coded as such. The risk factors listed above were compared in these two groups. Management of shoulder dystocia and its relationship to fetal outcome and maternal morbidity were analyzed as well.

Material and methods

All vaginal deliveries (10,662 cases) performed at the Toronto General Hospital from April 1980 to March 1985 were analyzed retrospectively. There were 91 deliveries coded for shoulder dystocia; 24 were cases of true shoulder dystocia (group 1); and the remaining 67 cases, although coded as shoulder dystocia, merely required increased traction for delivery (group 2). Risk factors for shoulder dystocia were compared in both groups, with prolonged second stage referring to >1½ hours for a multiparous patient and >2 hours for a primiparous one.[7] Maternal and fetal outcome were also compared. Fetal morbidity included the presence of one of the following: fractures, neurological damage such as palsies, and Apgar scores of <5 at 5 minutes. Maternal morbidity refers to the presence of uterine rupture, postpartum hemorrhage, and fourth-degree lacerations of the vagina.

The Fisher exact test and χ^2 with the Yates correction were used for statistical analysis.

Results

The risk factors for shoulder dystocia in both groups are described in Table 1. There was no significant difference in average weight or percentage of macrosomia between the two groups. Diabetes was present in only one patient in group 2 in a case with induction at 38 weeks and delivery of a 3880 gm infant. There was no significant difference in the risk factors related to macrosomia between the two groups. Midforceps delivery and a prolonged second stage were more common in group 1 compared to group 2; however, the difference between the two groups was not statistically significant. Although the combination of these two risk factors was 3.5 times more common in group 1 (21%) versus group 2 (6%), this difference did not prove to be statistically significant.

The neonatal and maternal complications are given in Table II. The morbidity associated with true shoul-

From the Division of Maternal and Fetal Medicine, Toronto General Hospital and The Perinatal Complex, University of Toronto.
Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.
Reprint requests: Dr. J. Shime, Department of Obstetrics and Gynecology, Toronto General Hospital, 200 Elizabeth St., Toronto, Ontario, Canada M5G 2C4.

Table I. Risk factors in true and coded shoulder dystocia

Risk factor	True shoulder dystocia (n = 24)*		Coded shoulder dystocia (n = 67)†	
	n	%	n	%
Macrosomia				
Multiparity	15	63	38	57
Previous macrosomia	2	8	11	16
Obesity	4	17	6	9
Diabetes	0	0	1	1
Postterm	2	8	9	13
Prolonged second stage	6	25	8	12
Midforceps delivery	12	50	20	30
Prolonged second stage and midforceps delivery	5	21	4	6

*Average weight ± SD = 4069 ± 277 gm.
†Average weight ± SD = 3979 ± 472 gm.

der dystocia (group 1) was 42% (10 of 24 cases) as compared to 1.5% (one of 67 cases) in group 2 ($p < 0.005$). Morbidity in group 1 consisted of six cases of Erb's palsy, five fractured clavicles, and one respiratory arrest that was successfully recuscitated. In two cases the neonate had sustained more than one type of injury. In group 2 there was only one neonatal complication of a low Apgar score at 5 minutes, which resolved by 10 minutes. There was only one case of maternal morbidity, a fourth-degree tear that occurred in group 1.

The relationship between the maneuvers performed and the fetal outcome is given in Table III. All orthopedic and neurological damage was associated with a combination of traction and fundal pressure. Delivery of the posterior shoulder and the corkscrew maneuvers were associated with complications in only one case, in which a series of maneuvers were attempted which resulted in the case of respiratory arrest mentioned above. The child was successfully resuscitated, and follow-up of more than a year failed to reveal any developmental abnormalities.

Comment

Because of the retrospective nature of this paper, there is room for error. Those cases designated as coded shoulder dystocia may in fact be true shoulder dystocia if maneuvers were not appropriately documented. However, rigorous comparison between doctors' and nurses' notes revealed a high degree of consistency which we feel should minimize this source of error.

Acker et al.[6] have determined protraction and arrest disorders to be significantly associated with shoulder dystocia. Likewise, Benedetti and Gabbe[3] have identified prolonged second stage combined with midforceps delivery as an intrapartum risk factor. Our study found

Table II. Neonatal and maternal complications of shoulder dystocia

Complications	True shoulder dystocia (n = 24)		Coded shoulder dystocia (n = 67)	
	n	%	n	%
Neonatal				
Low 5 min Apgar score	1	4	1	1.5
Neurological	6	25	0	0
Orthopedic	5	21	0	0
Maternal				
Fourth-degree tear	0	0	1	1.5

Table III. Maneuvers for shoulder dystocia and neonatal complications

Maneuver	No. of patients	Complications	
		n	%
Fundal pressure	13	10	77
Delivery of posterior shoulder	5	1*	20
Corkscrew	6	1*	17
Suprapubic	1	1*	

*Only one patient underwent a series of maneuvers (suprapubic, corkscrew, delivery of posterior shoulder) which resulted in fetal hypoxia.

the incidence of combined prolonged second stage with midforceps delivery to be considerably more common in true shoulder dystocia (ratio, 3.5:1). Although the Fisher exact test revealed a p value of 0.0508, the lack of significance with χ^2 with the Yates correction may be a reflection of the small numbers used for analysis.

Macrosomia is a well-documented risk factor for shoulder dystocia.[8] There was only one instance of diabetes in group 2, and it was not associated with macrosomia. This may reflect the practice of diabetic patients being delivered before 40 weeks, often by a cesarean delivery, and possibly a better control of diabetes in pregnancy. Macrosomia was not more common in the true shoulder dystocia group, which suggests that body configuration in macrosomia may be more important than macrosomia per se. This concept was suggested by Modanlou et al.,[5] who performed anthropometric studies post partum which revealed that macrosomic neonates with shoulder dystocia had a greater shoulder-head and chest-head disproportion regardless of birth weight. Their findings paralleled ultrasound studies in diabetic mothers in which a macrosomia index was calculated by subtracting biparietal diameter from chest diameter. Elliot et al.[9] suggested that an index >1.4 cm suggests shoulder dystocia. Such measurements may be indicated before labor if ultrasonographic estimated fetal weight is >4000 gm.

The morbidity rate of 42% was high but not incon-

sistent with other reports.[2, 3] The corkscrew maneuver and delivery of the posterior shoulder were associated with good fetal and maternal outcome. It is interesting that there was no documentation of hip flexion (McRoberts maneuver), which is reported to be simple and effective.[10] Fundal pressure in combination with traction was associated with neurological and orthopedic complications. The above findings would not be unexpected in light of the mechanism of fundal pressure which further impacts the anterior shoulder into the pubic bone, increases the risk of brachial plexus injury, and necessitates increased traction to accomplish the delivery. Furthermore, the use of this maneuver delays the implementation of more effective techniques.

Shoulder dystocia is a rare complication of labor that most obstetricians encounter infrequently. Management of shoulder dystocia is therefore often based on cumulative experience. A high index of suspicion should be maintained in the presence of certain risk factors. The present study also suggests that maneuvers such as the corkscrew maneuver or delivery of the posterior shoulder, which are mechanically logical, are safer than use of traction combined with fundal pressure, which only serves to compound the existing problem.

REFERENCES

1. Resnick R. Management of shoulder girdle dystocia. Clin Obstet Gynecol 1980;23:559.
2. Golditch IM, Kirkman K. The large fetus—management and outcome. Obstet Gynecol 1978;52:26.
3. Benedetti TJ, Gabbe SG. Shoulder dystocia—a complication of fetal macrosomia and prolonged second stage of labor with midpelvic delivery. Obstet Gynecol 1978; 52:526.
4. Modanlou HD, Dorchester W, Thorosian A, Freeman RK. Macrosomia: maternal, fetal and neonatal implications. Obstet Gynecol 1980;55:420.
5. Modanlou HD, Komatsu G, Dorchester W, Freeman RK, Bosu SK. Large-for-gestational-age neonates: anthropometric reasons for shoulder dystocia. Obstet Gynecol 1982;60:417.
6. Acker DB, Sachs BP, Friedman EA. Risk factors for shoulder dystocia in the average-weight infant. Obstet Gynecol 1986;67:614.
7. Zuspan FP, Quilligan EJ. Practical manual of obstetrical care. St. Louis: C. V. Mosby, 1982.
8. Harris BA. Shoulder dystocia. Clin Obstet Gynecol 1984; 27:106.
9. Elliot JP, Garite TJ, Freeman RJ, McQuown DS, Patel JM. Ultrasonic prediction of fetal macrosomia in diabetic patients. Obstet Gynecol 1932;60:159.
10. Gonik B, Stringer CA, Held B. An alternate maneuver for management of shoulder dystocia. Am J Obstet Gynecol 1983;145:882.

Cervical ripening and labor induction with prostaglandin E_2 gel: A placebo-controlled study

Paul Bernstein, M.D., Nicholas Leyland, M.D., Paul Gurland, M.D., and Douglas Gare, M.D.

Toronto, Ontario, Canada

A randomized double-blind, placebo-controlled study was undertaken to evaluate the effect of a single intracervical application of prostaglandin E_2 gel on the ripening of the cervix and on the subsequent induction of labor with oxytocin in patients with low Bishop scores (≤ 4). Compared to controls receiving gel only, the group receiving prostaglandin E_2 gel had significant increases in their cervical Bishop scores, shorter induction-to-delivery intervals, shorter time requiring use of oxytocin, and more successful labor induction without oxytocin. Systemic side effects were minimal and fetal outcomes were comparable, as were the routes of delivery. (Am J Obstet Gynecol 1987;156:336-40.)

Key words: Induction of labor, cervical ripening, prostaglandin E_2

From the Departments of Obstetrics and Gynecology, Mount Sinai Hospital and Toronto General Hospital, University of Toronto.
Supported by The Upjohn Company, Toronto, Ontario, Canada, and Kalamazoo, Michigan.
Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.
Reprint requests: Dr. Paul Bernstein, 600 University Ave., No. 467, Toronto, Ontario, Canada M5G 1X5.

Spontaneous labor and vaginal delivery in uncomplicated parturition follow a cascade of synchronous events that include the softening and effacement of the cervix. If this "ripening" of the cervix fails to occur, it may be accurately predicted that attempts at induction will be prolonged if not completely unsuccessful.[1]

Various complications of pregnancy may necessitate

delivery before the cervix has undergone the ripening process. This may serve to impede attempts at induction and predispose patients to increased fetal and maternal morbidity.

A number of clinical trials have supported the efficacy of prostaglandin E_2 in facilitating subsequent attempts of the induction of labor.[2,3] Of all the reported routes of prostaglandin administration, the direct application of the agent into the cervix appears to be the most advantageous in terms of increased efficacy and diminished side effects.[4]

The objective of this study was to determine, in comparison to placebo application, the effect of a single intracervical dose of prostaglandin E_2 gel on cervical ripening and the subsequent induction of labor with oxytocin.

Material and methods

Women who presented with medical and/or obstetric indications for the induction of labor were enrolled in the study from the Mount Sinai and Toronto General Hospitals. Ultimately 107 women were included in the analysis.

Admission criteria included written informed consent before entry into the trial, a gestational age of ≥36 weeks, Bishop score of ≤4, and parity of ≤3.

Exclusion criteria included known hypersensitivity to prostaglandins, any previous attempt at the induction of labor or cervical ripening, previous uterine surgery, suspected or clinically evident fetal compromise, vaginal bleeding, ruptured membranes, and fetal death in utero.

Patients were then randomized in a double-blind fashion to the prostaglandin E_2 group (n = 52) or the control group receiving the placebo (n = 55). On admission to the hospital, the patients were observed for 30 minutes during which time a nonstress test was performed, maternal vital signs were monitored, and the initial laboratory tests were obtained. These included a complete blood count and differential, renal, and liver function tests and urinalysis.

If no abnormalities were detected and the inclusion criteria were met, the initial Bishop score was assigned (Table I). After this, under direct cervical visualization, either 0.5 mg of prostaglandin E_2 in 2.5 ml of gel or a placebo administration of 2.5 ml of gel only was injected by means of a prepared syringe and a sterile 2.5 mm Portex catheter into the cervical canal to the level of the internal os.

The patients then remained in the delivery suite where cardiotocographic monitoring was performed continuously for 1 hour and intermittently thereafter. Maternal vital signs were recorded every 15 minutes for 1 hour, every hour for 3 hours, and every 4 hours thereafter.

Patients who went into labor were managed and monitored by their attending obstetrician as deemed appropriate. Labor was defined as the onset of regular uterine contractions resulting in the effacement and dilatation of the cervix to ≥3 cm.

If labor did not ensue by reassessment at 12 hours (t_{12}), a second Bishop score was assigned by the same observer and oxytocin induction was commenced with a use of 10 U of oxytocin per liter of ⅔:⅓ solution beginning at a rate of 2 mu/min, doubling the concentration every 15 to 20 minutes as necessary. If labor commenced, each case was managed individually by the patient's obstetrician. In those cases where no active labor was detected after 12 hours of oxytocin administration, the decision to continue or to cease induction attempts was made by the attending physician. Patients were followed as to outcome and number of induction attempts, and the results are given in the following section.

Statistical analysis consisted of Wilcoxon, Mann, and Whitney rank sum analysis, and in addition, an analysis of variance technique was used to assess the influence parity on the outcome parameters.

Table I. Cervical scoring chart

	Score		
	0	*1*	*2*
Dilatation (cm)	0	1-2	3-4
Effacement (%)	0-30	40-50	60-70
Station	−3	−2	−1/0
Consistency	Firm	Medium	Soft
Position of cervix	Posterior	Middle	Anterior

Table II. Comparison of study groups

	Prostaglandin E_2 group (n = 52)	*Placebo group (n = 55)*
Age (yr)	29.3 ± 5.7	29.7 ± 4.5
Weight (kg)	75.7 ± 12.7	77.5 ± 15.6
Gestational age (wk)	40.95 ± 1.6	41.09 ± 1.39
Gravidity	1.76 ± 1.0	1.76 ± .90
Mean arterial pressure (mm Hg)	92.0 ± 11.5	89.25 ± 10.0
Parity	0.35 ± 0.59	0.41 ± 0.79

Results

Table II illustrates that the prostaglandin E_2 and placebo groups were comparable in terms of age, weight, gestational age, gravidity, mean arterial pressure, and initial Bishop scores (t_0).

A comparison of the indications for induction of labor (Table III) reveals the two groups to be similar with the most frequent induction being postdates.

Table IV summarizes the outcomes of the various phases of the study. As can be seen, the two groups began the study with similar Bishop scores (t_0); however, the scores 12 hours after cervical application (t_{12})

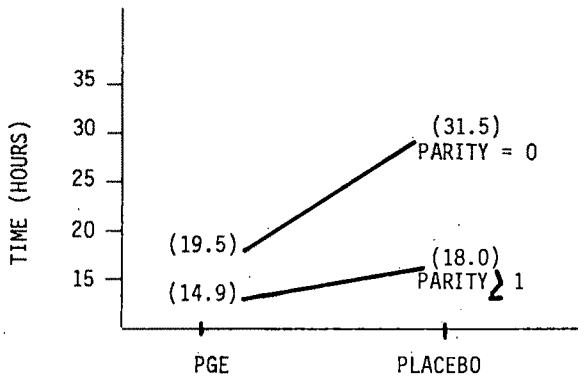


Fig. 1. Time (in hours) from t_0 to delivery. Effect of parity is illustrated. Both multiparous and primiparous women required significantly less time after prostaglandin E_2 *(PGE_2)* administration than after placebo administration, although multiparous women demonstrated a more pronounced effect.

Table III. Reasons for induction

	Prostaglandin E_2 group	*Placebo group*
Postdates	32	40
Preeclampsia/hypertension	9	6
Renal disease	1	0
Urticaria	0	1
Intrauterine growth retardation	3	1
Oligohydramnios	1	2
Polyhydramnios	1	0
Previous stillbirth	0	2
Isoimmunization	3	0
Macrosomia	1	0
Diabetes	0	3
Repeat false labor	1	0
Sample size	52	55

reveal a statistically significant increase in the prostaglandin E_2 scores ($p < 0.005$). In addition, a comparison of the net changes in Bishop scores (t_{12}-t_0) is also significant ($p < 0.0007$).

In a comparison of all patients, the time in hours from t_0 to complete dilatation and to delivery were both significant ($p < 0.0001$ and $p < 0.0002$, respectively).

Of those patients requiring oxytocin for induction (prostaglandin E_2 group, n = 22; placebo group n = 45), the time from induction to delivery was significantly lower in the prostaglandin E_2 group ($p < 0.05$) (Fig. 1).

The time from amniotomy to delivery and the maximum dose of oxytocin did not achieve statistical significance. The placebo group received oxytocin for purposes of induction for a greater length of time ($p < 0.03$).

Of the 52 patients receiving prostaglandin E_2 gel, 32 (60%) achieved labor; of these, 17 (33%) were delivered without requiring oxytocin. In the control group eight of 55 (15%) went into labor and six (11%) were delivered without receiving oxytocin.

Table V uses analysis of variance to clarify the role of parity on the outcome parameters found to be significant. For the parameters of time t_0 to delivery and t_0 to full dilatation, both parity and group effects (prostaglandin E_2 or placebo) are significant. In other words, the gel is effective in nulliparous and multiparous women, but it appears to have a greater effect in the former. There is no interaction between the group or parity effect.

For the subpopulation of patients which excludes those in whom labor began before oxytocin induction, no significant parity or group effect or interaction is demonstrated. This would indicate that for those patients not achieving labor within 12 hours of gel insertion, the effects on the subsequent induction of labor are less significant (Table VI).

There were no significant differences in mode of delivery (Table VII). The cesarean section rate in the prostaglandin E_2 group was 30.7% compared to 24% in the placebo group. This was not statistically significant. The indications for cesarean section are listed in Table VIII.

Laboratory parameters before and after the study did not change in either patient groups significantly. Maternal peripartum morbidity was minimal in the two groups. No significant side effects resulting from prostaglandin administration were observed. Previous series have reported systemic side effects such as fever, nausea, diarrhea, and uterine hypertonus. There were two cases of increased uterine activity in the period immediately after gel insertion. This resulted in variable decelerations in one case that responded to repositioning the patient, oxygen, and intravenous fluid bolus. The fetal heart rate tracing became normal. In the second case there was persistent fetal tachycardia and fetal heart rate decelerations. Delivery by cesarean section was accomplished 2 hours after gel insertion on the decision of the attending obstetrician. Both infants had Apgar scores of 8 and 9 at birth.

Fetal outcomes with regard to weight and Apgar scores at 1 and 5 minutes, respectively, were comparable between the two groups (Table IX).

In the group receiving placebo (gel only), three patients had evidence of tetanic contractions at the onset of oxytocin the next morning. In all three the uterus was allowed to relax and the induction resumed after a short time interval. Thus it is apparent that in both modes of induction the presence of hypertonic uterine activity may pose a problem.

Comment

On review of the literature, several studies have been performed evaluating the efficacy of prostaglandin E_2 in facilitating or initiating labor.[5, 6] Various routes

Table IV. Study parameters for prostaglandin E_2 and placebo groups and significance levels

	Prostaglandin E_2 group (n = 52)	*Placebo group (n = 55)*	*p Value*
Bishop score at t_0	2.84 ± 1.09	2.90 ± 0.91	NS
t_{12}*	6.57 ± 2.65	5.07 ± 2.46	<0.005
$t_{12} - t_0$	3.82 ± 2.56	2.13 ± 2.16	<0.0007
Time to complete dilatation (hr)†	15.39 ± 5.85	25.52 ± 15.82	<0.0001
Time to delivery from t_0 (hr)	19.00 ± 12.90	29.30 ± 21.90	<0.0002
Induction to delivery (hr)‡	12.30 ± 16.50	18.30 ± 19.60	<0.05
Rupture of membranes to delivery (hr)	6.30 ± 3.73	6.90 ± 3.74	NS
Maximum dose of oxytocin	18.00 ± 13.40	18.00 ± 11.40	NS
Total time of oxytocin administration (hr)	7.76 ± 3.35	11.70 ± 9.11	<0.03
Labor before use of oxytocin (n)	32	8	<0.0001
Delivery before use of oxytocin (n)	17	6	<0.0059

Analysis by Wilcoxon, Mann, and Whitney rank sum test.
*If in active labor or after delivery, maximum score = 10.
†Prostaglandin E_2 gel group, n = 37; placebo group, n = 40.
‡Prostaglandin E_2 gel group, n = 22; placebo group, n = 45.

Table V. Effect of parity on induction parameters

	Total population		
Time period	*Parity*	*Group*	*Interaction*
Induction to delivery	NS	NS	NS
Delivery from t_0	<0.05	<0.05	NS
Dilatation from t_0	<0.05	<0.01	NS
Rupture of membranes to delivery	<0.001	NS	NS

Friedman's two-factor analysis of variance.

Table VI. Effect of parity on induction parameters excluding patients in labor at t_{12}

Time period	*Parity*	*Group*	*Interaction*
Induction to delivery	NS	NS	NS
Delivery from t_0	NS	NS	NS
Dilatation from t_0	NS	NS	NS
Rupture of membranes to delivery	0.05	NS	NS

Friedman's two-factor analysis of variance.

Table VII. Mode of delivery

	Prostaglandin E_2 gel group (n)	*Placebo group (n)*	*p Value*
Spontaneous	15	15	NS
Low forceps	11	15	NS
Midforceps	10	12	NS
Cesarean section	16	13	NS

Table VIII. Indications for cesarean section

Indication for cesarean section	*Prostaglandin E_2 group*	*Placebo group*
Fetal distress	4	2
Disproportion/failure to progress	11	8
Breech presentation	0	1
Failed induction	0	2
Unripe cervix	1	0

Table IX. Fetal outcome

	Prostaglandin E_2 group	*Placebo group*	*p Value*
Fetal weight (gm)	3425 ± 542	3755 ± 510	NS
Apgar score, 1 min	8.01 ± 1.2	8.10 ± 1.3	NS
Apgar score, 5 min	9.0 ± 0.5	9.1 ± 0.4	NS

of administration (intravaginal, intracervical), variable forms of administration (tablets, gels), and varying dosages and dosage schedules have been used.[7, 8]

As stated in the introduction, our goal is to determine a safe and reliable method of ensuring that an indicated induction proceeds smoothly for both mother and fetus. Widespread acceptance of prostaglandin E_2 has met with some resistance because of unpredictable absorption and concerns regarding systemic toxicity and uterine hypertonia.

In more recent reports it would appear that lower doses of prostaglandin E_2, such as the 0.5 mg used in the study, can be both safe and efficacious. Clinical research in this area has been hampered by the lack of a stable preparation of uniform dose. The product used in the study was prostaglandin E_2, 0.5 mg, stabilized in a viscous methylcellulose gel supplied by the Upjohn Company of Canada.

In this study, as in previous reports,[2, 3] a single-dose regimen given in a double-blind, placebo-controlled fashion facilitated cervical ripening, and in addition, by itself induced labor in a significant proportion of patients. For those patients not achieving labor, the subsequent induction of labor was facilitated by the preinduction prostaglandin application. The drug appears to be free of major side effects for mother or fetus. It

must be stressed, however, that this trial was carried out under strict surveillance techniques.

Prostaglandin E_2 gel does induce uterine contractility and may occasionally result in uterine hypertonia. Because the conditions that most frequently necessitate induction, that is, postdates, hypertensive disorders of pregnancy, intrauterine growth retardation, etc., may be associated with placental insufficiency, the "ripening" period must include careful fetal monitoring throughout the entire latent period but especially in the first 1 to 2 hours. It is therefore our opinion that this drug can be safely used in any setting in which a cesarean section can be accomplished quickly if indicated.

In some studies reviewed there is the suggestion that use of an effective ripening agent might reduce the overall incidence of cesarean section.[9] In this study both groups had statistically comparable rates of cesarean section; however, it is of interest that few if any were due to "failed induction," so one might conclude that any approaches previously including "failed induction" as an indication for cesarean section will definitely benefit from the use of prostaglandin E_2 gel.

Some questions, of course, remain unanswered. It is still not clear as to what is the optimal preinduction period after administration of a single dose of prostaglandin E_2 gel (4, 8, or 12 hours). Additionally, the potential benefit of supplementary doses for those persistently unfavorable cervices needs to be evaluated. Indeed studies are now being carried out to assess low-dose repetitive cervical applications of prostaglandin E_2 gel.

In summary, a single low-dose intracervical application of prostaglandin E_2 gel proved to be a safe and reliable aid in dealing with indicated but potentially difficult inductions. The product has the advantages of being uniformly prepared, accurate in dosage, stable, easy to apply, and associated with minimal side effects for mother or fetus.

We wish to thank the Upjohn Company of Canada for their support of this project and also to thank the case room nurses whose efforts made the study possible.

REFERENCES

1. Prins RP, Bolton RN, Mark C, et al. Cervical ripening with intravaginal prostaglandin E_2 gel. Obstet Gynecol 1983; 61:459.
2. Yonekura MD, Songster G, Smith-Wallace T. Preinduction cervical priming with PGE_2 intracervical gel. Am J Perinatol 1985;2:305.
3. Trofatter KF, Bowers D, Gall SA, Killam AP. Preinduction cervical ripening with prostaglandin E_2 gel. AM J OBSTET GYNECOL 1985;153:268.
4. Ulmsten U, Wingerup L, Ekmar G. Local application of prostaglandin E_2 for cervical ripening or induction of term labor. Clin Obstet Gynecol 1983;26:95.
5. Theiry M. Preinduction cervical ripening. Obstet Gynecol Annu 1983;12:103.
6. Ulmsten U, Wingerup L, Bolfrage P, et al. Intracervical application of prostaglandin gel for induction of term labour. Obstet Gynecol 1982;59:336.
7. Laursen HN, Wilson KH. Induction of labour with oral prostaglandin E_2. Obstet Gynecol 1974;44:793.
8. Varma TR, Norman J. A comparison of three dosages of prostaglandin E_2 pessaries for ripening the unfavourable cervix prior to induction of labour. Acta Obstet Gynecol Scand 1984;63:17.
9. Nimrod C, Currie J, Yee J. et al. Cervical ripening and labour induction with intracervical triacetin based prostaglandin E_2 gel. A placebo-controlled study. Obstet Gynecol 1984;64:476.

Clinical utility of multiple-dose administration of prostaglandin E_2 gel

Thomas Mainprize, M.D., Carl Nimrod, M.B., Gail Dodd, R.N., and David Persaud, M.Sc.
Calgary, Alberta, Canada

An open-label study was undertaken to assess the clinical impact of multiple doses of intracervical prostaglandin E_2 (0.5 mg) gel administered at 6-hour intervals. Fifty women with low Bishop scores requiring induction of labor were recruited. The prestudy mean Bishop score was 2.3 ± 1.1 and changed significantly with one ($p < 0.001$), two ($p < 0.001$), and three ($p < 0.002$) doses. The mean gestational age of patients receiving three doses was significantly less than that of patients receiving one dose, (38.5 versus 40.1 weeks, $p < 0.005$). Prostaglandin E_2 gel induced labor in 56% of patients, but 14% required subsequent oxytocin (Syntocinon) augmentation. Cesarean section was performed in 6% of patients. No deleterious fetal, neonatal, or maternal effects occurred. (Am J Obstet Gynecol 1987;156:341-3.)

Key words: Obstetrics, prostaglandin E_2, induction of labor

Prostaglandins are effective in ripening the unfavorable cervix and inducing labor.[1-3] The low incidence of side effects, especially gastrointestinal, has made the intravaginal and intracervical routes of administration popular.[4, 5]

Ripeness of the cervix influences the success of induction, thus the patient with an unfavorable cervix represents a greater challenge when induction is deemed necessary.[6] In a previous study,[5] we demonstrated that single-dose intracervical prostaglandin E_2 (PGE_2) was efficacious in changing the Bishop score and inducing labor in a double-blind placebo-controlled situation. In that study, however, 40% of the patients receiving PGE_2 did not go into labor and a significant proportion of these did not achieve cervical ripeness during the 12-hour observation period. It seemed reasonable, therefore, to consider multiple gel applications in situations in which a satisfactory result was not achieved with a single dose and, from a practical standpoint, reduce the interval of gel application from 12 to 6 hours.

This study was therefore designed to assess the clinical utility and patient safety of a protocol that allowed the administration of multiple doses of intracervical PGE_2 in women with low Bishop scores requiring induction.

From the Division of Perinatal Medicine, Department of Obstetrics and Gynaecology, University of Calgary, and Foothills Provincial General Hospital.

Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.

Reprint requests: Dr. Thomas Mainprize, Department of Obstetrics and Gynaecology, Division of Perinatal Medicine, Foothills Hospital, 1403-29th St. N.W., Calgary, Alberta T2N 2T9 Canada.

Table I. Indications for induction of labor

	n	%
Postmaturity	20	40
Pregnancy-induced hypertension	14	28
Intrauterine growth retardation	6	12
Social	3	6
Maternal conditions	3	6
Fetal compromise	2	4
Diabetes	2	4

Material and methods

This study was an open-label design involving multiple administration of PGE_2 gel to a maximum of three doses at 6-hour intervals. The study population of 50 patients was drawn from patients admitted by their attending physician for induction of labor at Foothills Provincial General Hospital. Patients were included if they had gestation ages of ≥36 weeks, a parity of 0 to 3, a modified Bishop score of ≤4, and an expected vaginal delivery of a healthy newborn infant. Patients who had contraindications to uterine stimulation were excluded.

Bishop score was determined and informed consent obtained. Laboratory evaluation, including complete blood count and urinalysis, was made before initial administration and repeated within 48 to 72 hours after the last dose.

After a 30-minute evaluation period, 0.5 mg of PGE_2 in 2.5 ml of tri-acetin gel was administered into the cervical canal via a nylon catheter. Direct visualization of the cervix obtained by use of a speculum enabled the investigator to reach the internal os and withdraw the catheter slightly. Allowances were made for the catheter volume by an overfill in the syringe. The catheter was removed directly after administration and the

Table II. Data for primiparous patients (N = 24)

	One dose (0 hr)	*Two doses (6 hr)*	*Three doses (12 hr)*
Maternal age (yr)	27.33 ± 5.02	27.40 ± 6.19	32.75 ± 6.95
Gestational age (wk)	40.47 ± 1.55	39.00 ± 2.00	37.50 ± 1.73
Prestudy Bishop score	2.60 ± 0.99	2.00 ± 0.71	0.75 ± 0.96
Change in Bishop score	3.43 ± 1.75	3.83 ± 1.33	5.33 ± 2.52
Labor induced by gel	6	2	2
Labor induced by oxytocin	9	3	2
Oxytocin augmentation	3	0	0
Delivery outcome			
Vaginal	14	5	3
Operative	1	0	1

Table III. Data for multiparous patients (N = 26)

	One dose (0 hr)	*Two doses (6 hr)*	*Three doses (12 hr)*
Maternal age (yr)	27.53 ± 3.96	28.33 ± 4.93	32.83 ± 3.19
Gestational age (wk)	39.88 ± 1.50	39.67 ± 2.08	38.67 ± 1.63
Prestudy Bishop score	2.82 ± 0.95	3.00 ± 1.00	1.67 ± 1.03
Change in Bishop score	3.44 ± 2.31	1.67 ± 1.21	3.20 ± 2.28
Labor induced by gel	13	3	2
Labor induced by oxytocin	4	0	4
Oxytocin augmentation	4	0	0
Delivery outcome			
Vaginal	17	3	5
Operative	0	0	1

Table IV. Comparison of prestudy Bishop scores with 6- and 12-hour evaluations

	No. of cases	*Mean*	*p Value*
Prestudy	44	2.3 ± 1.0	<0.001
↓			
6 hr		5.5 ± 2.3	
Prestudy	12	1.5 ± 0.9	<0.001
↓			
12 hr		4.3 ± 1.3	

6-hour observation period was initiated. Detailed assessment of contractions and electronic monitoring of maternal and fetal health parameters were used throughout the study period.

Bishop score was determined 6 hours after initial administration by the same investigator if the patient was not in labor. For the purpose of this study, labor induction by gel was defined as the initiation of regular contractions with a dilation of the cervix of ≥3 cm. If the reassessed Bishop score was ≤4, a second dose of gel was given and a second 6-hour observation period initiated. The same procedure was performed at the end of the second 6 hours, and a 12-hour observation period followed the third dose. Subsequent labor induction with continuous intravenous oxytocin was commenced if the patient was not in labor either 12 hours after the third dose (24 hours after the initial dose) or after a Bishop score of >4 was achieved at the end of the observation period. Oxytocin was continued until delivery or for 12 hours, at which time the attending physician decided on further management. Augmentation of labor was determined by the investigator and amniotomy was withheld until labor was established.

Evaluation of labor, delivery, neonatal outcomes, and side effects were documented. Data were analyzed by χ^2 analysis.

Results

The indications for induction are summarized in Table I. Eighty percent of the study group fell into the categories of postmaturity, pregnancy-induced hypertension, and intrauterine growth retardation.

Tables II and III demonstrate data with respect to maternal age, gestational age, prestudy Bishop scores, change in Bishop scores, labor characteristics, and delivery outcomes for primiparous and multiparous women. Results showed the two groups to be comparable in all aspects and indicate that in situations with poor Bishop scores parity does not appear to affect the number of doses of gel required.

The mean change in Bishop scores from 0 to 6 hours was 3.4 ± 1.7 for primiparous women and 3.4 ± 2.3 for multiparous women, showing no statistically significant difference. There appeared to be a trend toward a higher mean change in Bishop score from 6 to 12

hours in the primiparous women; however, a statistically significant difference was not achieved. Comparison of the prestudy Bishop score with 6- and 12-hour evaluations is shown in Table IV. A statistically significant difference was achieved and illustrates the efficacy of intracervical PGE_2 gel in affecting Bishop score.

Of the study population, 28 (56%) of the patients went into spontaneous labor, and seven of them required oxytocin augmentation. Surgical amniotomy was performed in 25 patients after labor was established.

Delivery outcomes showed that 47 (94%) of the patients achieved vaginal delivery. Three cesarean sections were performed because of cord proplapse, fetal distress, and failure to progress. The patient with failure to progress had a prestudy Bishop score of 0, which did not change despite three doses of PGE_2 gel.

Newborn Apgar scores were unaffected by the number of doses received, and 49 (98%) of the newborn infants were discharged after an uncomplicated hospital course. One neonate delivered by cesarean section because of cord prolapse was admitted to the neonatal intensive care unit for evaluation with a diagnosis of diaphragmatic hernia.

Postpartum hemorrhage occurred in three patients. Estimated loss was >500 ml; however, all cases were managed conservatively without the need for transfusion.

Comment

Data obtained in this study demonstrate the effectiveness of multiple-dose administration of PGE_2 gel at 6-hour intervals in changing Bishop scores and subsequent induction of labor irrespective of parity.

A higher mean gestational age in patients requiring one dose of gel might possibly contribute to a higher prestudy Bishop score in these patients and further indicate that Bishop score appears to be the more important variable in successful induction.

Forty-seven (94%) of 50 patients achieved vaginal delivery. A 6% cesarean section rate compares favorably with lower rates seen after prostaglandin priming compared with placebo.[7]

More rapid absorption of PGE_2 from cellulose gel rather than from tablets was demonstrated by Gordon-Wright.[8] An increased frequency of side effects might be anticipated with gel, but this did not occur in our study. Indeed, no adverse fetal or maternal effects could be attributed to PGE_2. Two cases of fetal distress were not associated with hypertonic uterine contractions; cord prolapse was diagnosed in one case. Nausea, when it occurred, was related to the use of narcotics for analgesia. The 6-hour interval between doses may reduce potential side effects from drug accumulation; however, this needs further study.

The incorporation of a protocol allowing repeated application of PGE_2 gel at 6-hour intervals in patients with an unfavorable cervix was useful in achieving a successful induction of labor and a low cesarean section rate and was unaffected by the parity of the patient. The absence of side effects in this study confirmed the safety of this regimen.

REFERENCES

1. Floberg J, Allen J, Belfrage P, Bygdeman M, Ulmsten U. Experience with an industrially manufactured gel PGE_2 for cervical priming. Arch Gynecol 1983;233:225.
2. Mackenzie IZ, Embrey MP. The influence of preinduction vaginal prostaglandin E_2 gel upon subsequent labour. Br J Obstet Gynaecol 1978;85:657.
3. Ulmsten U, Wingerup L, Anderson KE. Comparison of prostaglandin E_2 and intravenous oxytocin for induction of labor. Obstet Gynecol 1979;54:581.
4. Ulmsten U, Wingerup L, Belfrage P, Ekman G, Wiqvist N. Intracervical application of prostaglandin gel for induction of term labor. Obstet Gynecol 1982;59:336.
5. Nimrod C, Currie J, Yee J, Dodd G, Persaud D. Cervical ripening and labor induction with intracervical triacetin base prostaglandin E_2 gel: a placebo-controlled study. Obstet Gynecol 1984;64:476.
6. Bishop EH. Elective induction of labor. Obstet Gynecol 1955;5:519.
7. O'Herlihy C, MacDonald H. Influence of preinduction prostaglandin E_2 vaginal gel on cervical ripening and labor. Obstet Gynecol 1979;54:708.
8. Gordon-Wright AP, Elder MG. The systemic absorption from the vagina of prostaglandin E_2 administered for the induction of labor. Prostaglandins 1979;18:153.

Immunosuppressor factor(s) produced by decidua-associated suppressor cells: A proposed mechanism for fetal allograft survival

Salim Daya, M.B., Ch.B.,* Kenneth L. Rosenthal, Ph.D., and David A. Clark, M.D., Ph.D.**

Hamilton, Ontario, Canada

Mechanisms that explain failure of rejection of the antigenic fetus by the mother appear to lie at the fetomaternal interface. Successful pregnancies have been correlated with the presence of decidua-associated suppressor cells. Supernatants from these cells were tested for their ability to suppress the proliferation of interleukin 2–dependent cells and were also subjected to high-performance liquid chromatography. Decidual supernatant was found to have peak suppressive activity at two molecular weights (43,000 and 21,000 daltons). The activity of decidual supernatant was directed specifically at interleukin 2–dependent cells and interfered with interleukin 2 action. The importance of this mechanism in graft rejection is well known. Decidual supernatant may thus prevent fetal allograft rejection by preventing the maternal cytotoxic effectors from receiving the interleukin 2–dependent help that they need to proliferate and remain actively cytolytic. (Am J Obstet Gynecol 1987;156:344-50.)

Key words: Decidua, fetal allograft survival, interleukin 2

The mammalian fetus may be viewed as a type of allograft that is capable of survival in an immunologically hostile host. The fetus itself bears transplantation antigens against which the maternal immune system can react. Priming against fetal alloantigens in vivo results in the formation of antipaternal cytotoxic reactivity which has no effect on fetal health or survival.[1] Alloimmunization in several strain combinations of female mice against paternal antigens does not result in antibody formation despite the presence of major histocompatibility complex differences between the mother and fetus.[1] Thus systemic immunosuppression by allospecific T suppressor cells or by "blocking" antibodies does not appear to be necessary for survival of the fetal allograft.

In contrast, allografts placed at the choriodecidual junction experience prolonged survival despite the presence of class I major histocompatibility complex antigens on some fetal trophoblast populations which also resist lysis by natural killer cells and cytotoxic T lymphocytes.[2] Fetal rejection, however, is associated with an infiltrate of maternal cytotoxic cells, which appear to first pass through or between the trophoblast without causing lysis of the latter.[3] Such rejection has also been associated with a local deficiency in the decidua of nonspecific suppressor cells.[3, 4] These suppressor cells appear to be activated or recruited by trophoblast cells. The immunologic success or failure of the fetal allograft, therefore, appears to be determined largely by events at the fetomaternal interface.

Two types of maternal suppressor cells have been isolated from the human uterine lining.[4] These cells suppress the ability of maternal T cells to proliferate in response to concanavalin A. A large, probably hormone-dependent suppressor cell has been isolated from the endometrium of normal menstrual cycles. The suppressive activity of this cell is seen only in the luteal phase and is absent in the proliferative phase. Maximal activity appears to be present at a time when implantation would normally occur. A second, smaller, and probably trophoblast-dependent suppressor cell has been isolated from the decidua of successful pregnancies. Its activity is present even in the first pregnancy, and it is seen to gradually decline as term approaches.

The mechanism of suppression by these human suppressor cells has not been determined. In this article we show that decidua-associated suppressor cells elaborate soluble factors that have immunosuppressive properties through their ability to block interleukin 2 action. The importance of this mechanism is discussed,

From the Departments of Obstetrics and Gynecology, Medicine, and Pathology, McMaster University.

This work was supported by a grant from the Physicians' Services Incorporated Foundation.

Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.

Reprint requests: Dr. Salim Daya, Department of Obstetrics and Gynecology, McMaster University, 1200 Main St. West, Hamilton, Ontario, Canada L8N 3Z5.

**Career scientist of the Ontario Ministry of Health, Canada.*

***Scientist of the Medical Research Council of Canada.*

since it is known that interleukin 2 is a key molecule involved in the activation and proliferation of cytotoxic T lymphocytes that are necessary to mediate allograft rejection.

Material and methods

Animals. Mice of C3H/HeJ and DBA/2 strains were purchased from the Jackson Laboratory, Bar Harbour, Maine. Four or five were housed per cage and allowed water and food ad libitum. Sprague-Dawley rats were purchased from Charles River Laboratories, St. Constant, Quebec.

Cell preparation. All cells were resuspended in α–minimal essential medium supplemented so as to contain 10% fetal bovine serum, 100 μg/ml of penicillin, 100 μg/ml of streptomycin, 20 mmol/L of Hepes, sodium bicarbonate and 2.92 mg/100 ml of L-glutamine. Preparation included the following steps: (1) Peripheral blood lymphocytes were separated from venous blood by density centrifugation with use of Isopaque-Ficoll as previously described.[4] (2) Decidual lymphocytes were separated from decidua obtained by uterine curettage after elective abortion in the late first trimester. The lymphoid cells were separated by density centrifugation after single cell suspensions of the material were made as previously described.[4] (3) Endometrial lymphocytes were obtained by dilatation and curettage in the luteal phase from women with normal menstrual cycles undergoing infertility assessment. The lymphocytes were prepared in a similar way to decidual lymphocytes. (4) Peripheral lymph node lymphocytes were obtained from 6- to 8-week-old virgin C3H/HeJ female mice. (5) P815 (H-2d) mastocytoma cells were serially propagated in ascites in DBA/2 male mice. (6) YAC-1 lymphoma cells were cultured in vitro in RPMI supplemented to contain 10% fetal calf serum, 1% L-glutamine, 1% penicillin and streptomycin, 1% Hepes, and 1% sodium bicarbonate. (7) OW-7 human ovarian carcinoma cells (provided by Dr. R. N. Buick, Ontario Cancer Institute, Toronto, Canada) were grown in vitro in RPMI and disloged with use of trypsin before being resuspended in α-minimal essential medium.

Generation of supernatants. Preparations of 2×10^6 decidual lymphocytes and endometrial lymphocytes were each incubated in 1 ml of α-minimal essential medium for 48 hours at 37° C in 6% to 7% carbon dioxide. The cell-free supernatant was removed and stored at 4° C.

Purification of suppressor factor from crude decidual supernatant. Decidual supernatant was purified by high-performance liquid chromatography. A TSK gel G3000SW column (Toyo Soda USA Inc., Atlanta) that had been preequilibrated with Tris (0.01 mol/L) in sodium chloride buffer (0.10 mol/L) at pH 7.0 was used.

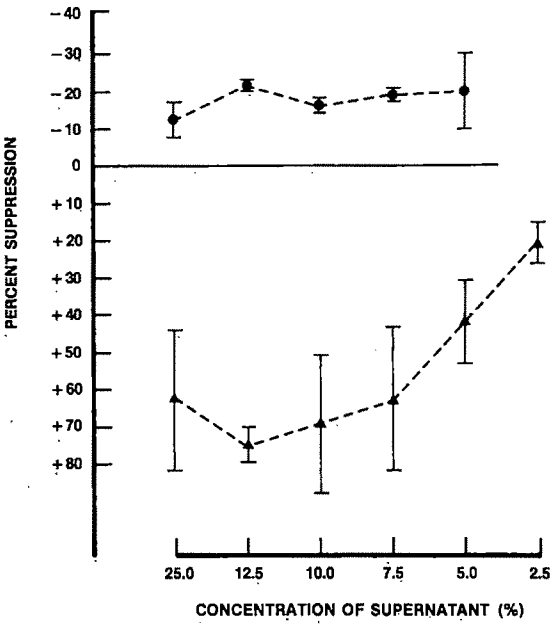


Fig. 1. Effect of endometrial and decidual supernatants on lymphocyte proliferation. Means ± SEM of three experiments showing the effect of various concentrations of endometrial supernatant (●) and decidual supernatant (▲) on concanavalin A–induced proliferation of human peripheral blood lymphocytes.

Fractions were eluted at a flow rate of 1 ml/min at room temperature. Each fraction was sterilized by filtration (Millex-GV 0.22 μm filter unit, Millipore Corporation, Bedford, Massachusetts) and then supplemented with fetal bovine serum to a final concentration of 10%. The column was calibrated with use of a variety of molecular weight markers, namely, β-amylase, alcohol dehydrogenase, bovine serum albumin, carbonic anhydrase, and ribonuclease.

Toxicity tests. Decidual supernatant was tested for toxic effect on lymphocytes by a comparison of the viability in 0.4% trypan blue of the cells cultured with use of decidual supernatant with the viability by means of medium alone.

Interleukin 2 responses. Interleukin 2 was prepared by concanavalin A stimulation of rat spleen cells with use of a standard method. Stated briefly, rat spleen cells at a density of 5×10^6 per milliliter were cultured in RPMI containing 5 gm/ml of concanavalin A; 48 hours later the supernatant was harvested after centrifugation at 1000 × g for 20 minutes. Supernatants were then sterilized by filtration and stored at −20° C until used. These interleukin 2 cells containing supernatants were tested for their ability to induce proliferation of H-Y cells, a cloned mouse natural killer cell line. This cell line has been shown to be interleukin 2–dependent with use of purified lymphokines (unpublished results). Twofold dilutions of interleukin 2 samples (at a final

Table I. Effect of decidual supernatant on proliferation of non–interleukin 2–dependent cells

Cells cultured	Tritiated thymidine incorporation	
	Cells cultured with medium alone (cpm)*.	Cells cultured with medium plus decidual supernatant (cpm)*†
YAC-1	532 ± 33	520 ± 54‡
P815	126982 ± 10167	126987 ± 5117‡
OW-7	5815 ± 643	6045 ± 561‡

*Fifty microliters per well of decidual supernatant was used to test for suppressive effect.

†Values represent mean ± SEM from four replicates of a typical experiment.

‡Not significantly different from incorporation in medium alone; $p > 0.05$.

concentration of 5%) were prepared, and 50 μl containing 1×10^4 H-Y cells was added to each well in 96-well, flat-bottomed microculture trays (Linbro, Flow Laboratories Inc., Mississauga, Ontario, Canada). At the beginning 100 μl of decidual supernatant was added to the interleukin 2 assay to test for its ability to block the interleukin 2–dependent proliferative response. Incubation was carried out at 37° C for 24 hours. The cells were then labeled with 1 mCi of tritiated thymidine (New England Nuclear, Boston) for 6 hours, harvested onto glass fiber strips, and processed for detection of radioactivity in a Beckman liquid scintillation counter.

Suppression assay. Peripheral blood lymphocytes, at a density of 1×10^5 cells per well were cultured in α-minimal essential medium and stimulated to proliferate with concanavalin A at a final concentration of 10 mg/ml. Suppressive activity of decidual supernatant (purified and crude) and crude endometrial lymphocyte supernatant were tested for their ability to suppress proliferation of the peripheral blood lymphocytes when compared with medium alone. The cells were cultured in 96-well, round-bottomed microculture trays for 48 hours followed by the addition of 1 mCi of tritiated thymidine. Incubation was carried out for a further 16 to 18 hours before harvest and detection of radioactivity. A similar assay was used for mouse peripheral lymph node lymphocytes. The tumor cell lines, however, were grown for the same duration but without the addition of concanavalin A. Time course experiments were also performed by use of peripheral blood lymphocytes and by varying the time when crude decidual supernatant was added to the cell cultures.

Statistical analysis. The degree of tritiated thymidine uptake in counts per minute (cpm) was used to determine suppression activity, which was calculated with use of the following formula:

$$\% \text{ Suppression} = \frac{(\text{cpm without supernatant} - \text{cpm with supernatant})}{(\text{cpm without supernatant})} \times 100$$

Results

Soluble suppressive activity. Fig. 1 shows the effect of supernatant from endometrial and decidual lymphocytes on mitogen-induced proliferation of human peripheral blood lymphocytes. The endometrial lymphocytes from the luteal phase of menstrual cycle are known to have a direct suppressive activity,[4] but the cells did not mediate suppression by producing a soluble suppressor factor (top of Fig. 1). In contrast, the supernatant from decidua-associated cells was highly suppressive even in very low concentrations (bottom of Fig. 1). This suggests that the suppressive activity produced by decidua-associated suppressor cells was mediated by elaboration of a potent soluble suppressor factor (or factors).

Mechanism of inhibition of proliferation. The possibility that suppression of peripheral blood lymphocytes proliferation by decidual supernatant might be the result of a nonspecific toxic effect was evaluated. Incubation of human lymphocytes with decidual supernatant for 24 hours at 37° C had no effect on viability (90.1% in medium versus 88.7% in decidual supernatant, $p > 0.05$). Decidual supernatant was also added during the final 18 hours of 3-day human lymphocyte cultures with concanavalin A when lymphoblasts were present. Blasts cultured in the presence of decidual supernatant had a viability of 75.4% whereas blasts grown in medium alone were 78.6% viable ($p > 0.05$). Thus the suppressive activity of decidual supernatant was not attributable to toxicity to cells during short-term (<24-hour) exposure. To determine whether toxicity occurred after a longer incubation period, decidual supernatant was included in 3-day cultures of three different non–interleukin 2–dependent cell lines (Table I). Proliferation was not inhibited, confirming that the suppressive activity of decidual supernatant was not due to a nonspecific toxic effect on the cell.

It is known that interleukin 2 induces activated cytotoxic T lymphocytes to proliferate, migrate, and carry out allograft rejection. The inhibition of lymphocyte proliferation by decidual supernatant could represent an interference with the generation or action of interleukin 2. To determine whether this occurred, decidual supernatant was added to cultures of three different interleukin 2–dependent cell lines (Table II). Significant suppression of proliferation occurred with all three cell lines, indicating that decidual supernatant inhibited interleukin 2–induced cell proliferation. Fig. 2 shows a direct dose effect of decidual supernatant,

Table II. Effect of decidual supernatant on proliferation of interleukin 2–dependent cells

Cells cultured	*Tritiated thymidine incorporation*	
	Cells cultured with medium alone (cpm)†	*Cells cultured with medium plus decidual supernatant (cpm)*†*
Human peripheral blood lymphocytes	64535 ± 2769	9399 ± 419
Mouse peripheral lymph node lymphocytes	586 ± 112	267 ± 78
H-Y cells	59524 ± 1998	29397 ± 7489

*Fifty microliters per well of decidual supernatant was used to test for suppressive effect.

†Values represent mean ± SEM from four replicates of a typical experiment.

demonstrating a marked reduction in H-Y cell proliferation with increasing concentrations of decidual supernatant.

Time course suppression by decidual supernatant. It is known that interleukin 2 is produced during the first 24 hours of culture and then binds to interleukin 2 receptors on activated lymphocytes. To determine whether decidual supernatant could antagonize the action of interleukin 2 *after* it had bound to receptors time course experiments were undertaken. Fig. 3 shows the effect of adding decidual supernatant to human lymphocyte cultures at a final concentration of 1:8 between 0 and 48 hours following initiation of concanavalin A stimulation. When decidual supernatant was added after 18 hours, there was no suppression. These data suggested that decidual supernatant acted on the cell or interleukin 2 before receptor interaction.

Size of suppressive molecules in decidual supernatant. To define more accurately the molecular species responsible for suppression by decidual supernatant, separation into different fractions was carried out by high-performance liquid chromatography. Fig. 4 shows that two major peaks of suppressive activity were obtained that corresponded to molecular weights of 43,000 and 21,000 daltons, respectively. There was also a smaller peak of suppressive activity corresponding to 60,000 daltons.

Comment

Successful pregnancies have been associated with the presence of suppressor cells in the uterine decidua.[3, 4] The mechanism of suppression by these cells in the human has not previously been determined. The data in this paper show that decidua-associated lymphocytes in early human pregnancy elaborate a soluble suppressor factor that acts by blocking the action of interleukin 2. This is clearly seen in cultures of H-Y cells which depend on interleukin 2 for proliferation and do not require the presence of an antigen. The soluble factor from decidua suppresses proliferation, suggesting that it interferes in some way with interleukin 2 action possibly by preventing interleukin 2 from binding to its receptor. The fact that the action of human decidual supernatant is directed specifically at interleukin 2–dependent cells is also shown by the observation of inhibition of mitogen-induced proliferation of both human lymphocytes and mouse peripheral lymph node cells. In contrast, the proliferation of three non–interleukin 2–dependent cell lines, including YAC-1 (a mouse lymphoma), P815 (a mouse mastocytoma), and OW-7 (a human ovarian carcinoma), was not inhibited.

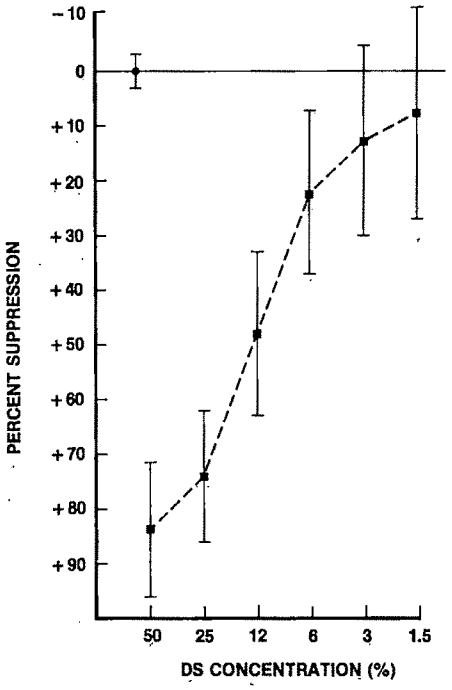


Fig. 2. Effect of decidual supernatant *(DS)* on interleukin 2–dependent cell proliferation. A 20% dilution of rat interleukin 2 was added to the interleukin 2–dependent H-Y cell line in the presence of decreasing concentrations of decidual supernatant (■) and in the presence of medium (●). Means ± SEM are shown.

The importance of interleukin 2 in graft rejection is well known. T helper cells become activated when presented with antigen and produce interleukin 2.[5] This augments the proliferation of alloantigen-specific cytotoxic T lymphocytes. It also activates and stimulates the proliferation of natural killer cells and a population of T cells (lymphokine-activated killer cells) that kill a variety of tumor cells that are resistant to specific cytotoxic T lymphocytes.[6-8] Thus a variety of cytotoxic effector cells can become activated through the effects of interleukin 2. This is necessary, since different types

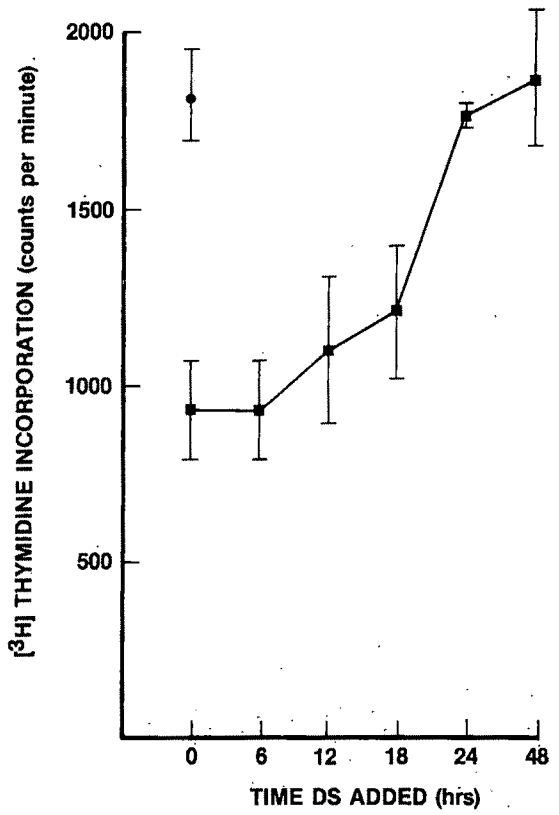


Fig. 3. Time course suppression by decidual supernatant. Decidual supernatant (■) at a final concentration of 12.5% was added to lymphocyte cultures at various times after initiation of concanavalin A stimulation. Medium (●) was used as control. Means ± SEM of three experiments are shown.

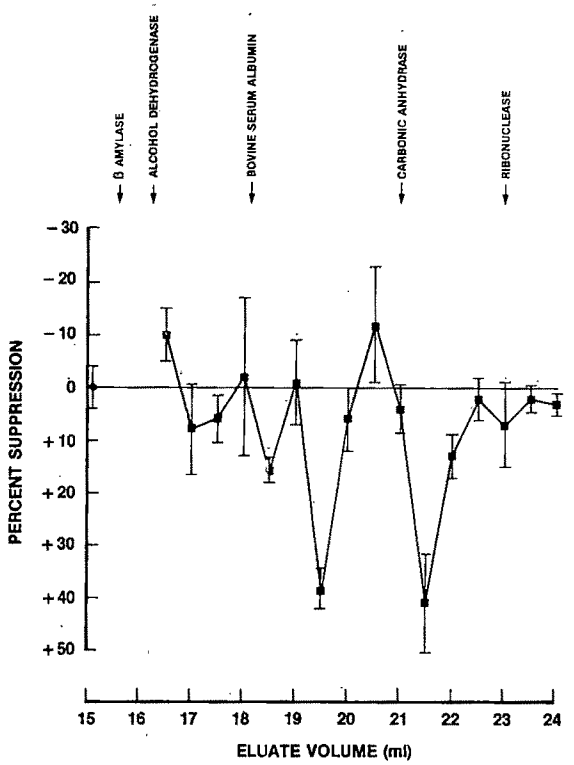


Fig. 4. Size of suppressive molecules in decidual supernatant. A 25% concentration of decidual supernatant was separated by high-performance liquid chromatography, and each fraction (■) was tested at a final concentration of 25% for suppression of concanavalin A–induced lymphocyte proliferation. Medium (●) was used as control. Means ± SEM are shown, as well as the molecular weight markers that were used for calibration.

of cells in a graft require different types of effector cells for their inactivation or destruction.[9] Antitumor effects of interleukin 2 have been determined by observing the rejection of nonimmune murine and human tumors into which it has been injected.[7, 10] Therefore it would seem that blocking the response to interleukin 2 could markedly affect the host's graft-destroying mechanisms. Decidual supernatant from decidua-associated suppressor cells may thus prevent fetal allograft rejection by preventing the maternal effectors from receiving the interleukin 2–dependent "help" that they need to proliferate and remain actively cytolytic. Since the motility of these effectors is interleukin 2–dependent, decidual supernatant may also block their migration into the fetus through the trophoblast.

There is some evidence to suggest that such suppressor activity may protect the antigen-bearing fetoplacental unit from adverse effects of maternal immunity. In situations of deficient suppressor cell activity in the uterus, the fetal allograft is highly susceptible to maternal antipaternal immunity. For example, in missed abortions in humans there is a deficiency of suppressor cell activity in the decidua.[4] Similarly a suppressor cell deficiency in decidua and in the lymph nodes draining the uterus correlates with the presence of spontaneous resorption of fetuses in allopregnant mice.[3] The inhibition of interleukin 2 responses by decidual supernatant would therefore be expected to protect fetuses from a variety of maternal effector mechanisms both in nonimmune animals and in women with transplantation immunity to paternal major histocompatibility complex antigens. Further studies are required to determine whether decidual supernatant can also inhibit some of these other effector cells such as natural killer cells, which kill embryonic cells and macrophages. This seems likely, since natural killer activity in the murine uterus decreases as pregnancy advances.[11]

The mechanism of activation of the decidua-associated suppressor cells is still under investigation. Decidual suppressor cells in the murine uterus are activated by trophoblast. Also recent studies of fractionated human luteal phase endometrial cells have shown that trophoblast membrane and soluble placental signals may be involved in this activation.[12] Therefore the fetus appears to be directly responsible for controlling its own survival in the mother. Failure to activate these

suppressor cells would then result in early pregnancy wastage.

The decidual supernatant factor was found to have a peak activity at two molecular weights, namely, 43,000 and 21,000 daltons, with a smaller peak of activity at 60,000 daltons. Whether these represent distinct molecules or are polymers or breakdown products is unclear. Decidual supernatant suppressor factor obtained from allopregnant mice (and which also blocks the response to interleukin 2) had a molecular weight of 80,000 to 100,000 daltons by sephacryl chromatography.[13] Recent studies with use of high-performance liquid chromatography methods have shown additional activities at 40,000 and 20,000 daltons (Clark et al., unpublished observations). Thus decidual supernatant appears to be similar in both the human and murine systems.

Other types of suppressor factors have been described in the literature. For example, Badet et al.[14] have obtained a 60,000 dalton factor from the decidua of syngeneically mated C3H mice as well as from deciduomas in which no trophoblast is present. Hardt et al.[15] described a T cell–derived factor in the serum of mice that inhibits the response to interleukin 2 and has a molecular weight of 50,000 daltons. Many tumor cells can also produce factors that suppress proliferation of T cells in vitro. These factors generally have molecular weights <50,000 daltons, and their mechanism of action has not yet been determined.

Other types of proteins produced by the human uterine lining have also been described in the literature. The functional properties of these proteins have not yet been determined. Progestogen-associated endometrial protein, a glycoprotein with a molecular weight of 47,000 daltons which can be reductively dissociated to two subunits with each having molecular weights of 27,000, has been isolated from human endometrium, and the amounts detected rise rapidly during early pregnancy.[16] Progestogen-associated endometrial protein appears to have a molecular weight similar to decidual supernatant, but it is not known whether it also has similar immunosuppressive properties. Other reports indicate that of the many secretory proteins of pregnancy endometrium, two appear more commonly during early pregnancy.[17] EP14 is a subunit with a molecular weight of 32,000 daltons that is associated with decidualized endometrium, and its rate of production increases during early pregnancy.[17] EP15, a subunit with a molecular weight of 28,000 daltons, is also produced by secretory endometrium during the menstrual cycle and during early pregnancy until 15 to 16 weeks' gestation after which it is no longer detectable. Again, no immunosuppressive activity has yet been detected with these proteins. Since skin grafts placed on deciduomas in parous immune mice are rejected whereas similar grafts at the choriodecidual junction are protected, trophoblast-dependent suppressor cells and their factors appear to be the major defense against rejection in vivo.[2]

It is rapidly becoming evident that fetal allograft survival against an active maternal immune system depends on a definable interaction of cells and immunomodulating factors at the fetomaternal interface. Development of monoclonal antibodies to the decidua-associated suppressor cell and its suppressor factor will provide more information about the intricate mechanism of this fetomaternal interaction. With this knowledge we may be able to develop a clearer understanding of the clinical problem of early embryo and pregnancy wastage and unexplained infertility. Ultimately we may be able to provide treatment for this chronic, frustrating, and distressing problem. One such treatment may be immunotherapy in women using allogeneic cells. This has been shown to boost decidual supernatant activity in mice, thereby preventing spontaneous abortion (Clark et al., unpublished observations).

We thank Rita Campbell for expert technical assistance.

REFERENCES

1. Chaouat G, Kolb JP, Wegmann TG. The murine placenta as an immunological barrier between the mother and the fetus. Immunol Rev 1983;75:31.
2. Sio JD. Allograft reactivity and progesterone involvement at the choriodecidual junction [Dissertation]. Dallas, Texas: University of Texas Health Sciences Center, 1985.
3. Clark DA. Maternofetal relations: a mini review. Immunol Lett 1985;9:239.
4. Daya S, Clark DA, Devlin C, Jarrell J. Preliminary characterization of two types of suppressor cells in the human uterus. Fertil Steril 1985;44:778.
5. Palacios R. Mechanism of T cell activation: role and functional relationship of HLA-DR, antigens and interleukins. Immunol Rev 1982;63:73.
6. Rosenstein M, Yron I, Kaufmann Y, Rosenberg SA. Lymphokine-activated killer cells: lysis of fresh syngeneic natural killer-resistant murine tumor cells by lymphocytes cultured in interleukin 2. Cancer Rev 1984;44:1946.
7. Forni G, Giovarelli M, Santoni A. Lymphokine-activated tumor inhibition in vivo. I. The local administration of interleukin 2 triggers nonreactive lymphocytes from tumor-bearing mice to inhibit tumor growth. J Immunol 1985;134:1305.
8. Gregory CT, Atkinson ME. Large granular lymphocytes: early non-specific effector cells in allograft rejection in the mouse. Immunology 1984;53:257.
9. Hayry P. Intragraft events in allopregnant destruction. Transplantation 1984;38:1.
10. Pizza G, Severini G, Menniti D, deVinca C, Corrado F. Tumor regression after intralesional injection of interleukin 2 (IL-2) in bladder cancer: preliminary report. Int J Cancer 1984;34:359.
11. Croy BA, Gambel P, Rossant J, Wegmann TG. Characterization of murine decidual natural killer (NK) cells and their relevance to the success of pregnancy. Cell Immunol 1985;93:315.
12. Daya S, Johnson PM, Clark DA. Mechanisms of activation

A complete list of references will be furnished on request to the authors.

of suppressor cells in the endometrium of the human uterus. J Reprod Immunol 1986;1(suppl):164.
13. Clark DA, Chaput A, Walker C, Rosenthal KL. Active suppression of host versus graft reaction in pregnant mice. VI. Soluble suppressor activity obtained from decidua of allopregnant mice blocks the response to IL-2. J Immunol 1985;134:1659.
14. Badet MT, Bell SC, Billington WD. Partial characterization of immunosuppressive factors from short-term cultures of murine decidual tissue. Ann Immunol (Paris) 1983;134:321.
15. Hardt C, Rollinghoff M, Pfizenmaier K, Mosmann H, Wagner H. Lyt-23+ cyclophosphamide-sensitive T cells regulate the activity of an interleukin-2 inhibitor in vivo. J Exp Med 1981;154:262.
16. Joshi SG. A progestagen-associated protein of the human endometrium: basic studies and potential clinical applications. J Steroid Biochem 1983;19:751.
17. Bell SC, Hales MW, Patel S, Kirwan PH, Drife JO. Protein synthesis and secretion by the human endometrium and decidua during early pregnancy. Br J Obstet Gynaecol 1985;92:793.

Fetal foot length as a predictor of gestational age

Brian M. Mercer, M.D., Suzi Sklar, R.D.M.S., Anahita Shariatmadar, M.Sc., Martin S. Gillieson, M.D., and Mary E. D'Alton, M.D.

Ottawa, Ontario, Canada

Ultrasonographic measurement of fetal foot length is useful in the assessment of gestational age. Two hundred twenty-three postpartum and 224 ultrasonographic measurements were performed between 11 and 43 weeks' gestation; 207 postpartum and 160 ultrasonographic measurements met our requirements of secure dates and no physical anomaly or maternal disease. Mean foot length at each week of gestation compared favorably with Streeter's data, based on pathologic specimens, described in 1920. Comparison of curvilinear regression of foot length versus gestational age demonstrated a strong correlation with an R^2 value of 0.981; 95% confidence intervals at each week compared favorably with both biparietal diameter and femur length data. Fetal foot length is a reliable parameter for use in the assessment of gestational age and is particularly useful when other parameters do not accurately predict gestational age, for example, hydrocephalus, anencephaly, short limb dysplasia. (Am J Obstet Gynecol 1987;156:350-5.)

Key words: Pregnancy, ultrasound, gestational age, foot length

With the development of obstetrical ultrasound as an adjunct to antenatal assessment, many parameters[1-3] have been studied in attempts to assess fetal gestational age. In 1920 Streeter[4] reported a series of 576 pathologic specimens in which he correlated foot length to gestational age. Measurements were taken from the heel to the first or second toe. Many specimens were fixed with 10% formalin, which may cause tissue swelling. Usher and McLean[5] in 1969 gave similar results with postpartum data measuring from the heel to the longest toe of normal neonates. There are no studies that have reviewed foot length and growth in anomalous infants, multiple gestation, or pregnancies with maternal medical complications.

It was our intent to confirm Streeter's and Usher and McLean's data with postnatal foot length measurements. We planned to compare antenatal ultrasonographic measurements with our postpartum findings and develop a nomogram for ultrasonographic foot length as a predictor of gestational age. Finally, we hoped to demonstrate the usefulness of foot length measurement in special clinical cases such as multiple gestation, infants of diabetic mothers, the anencephalic fetus, and the fetus with short limb dysplasia.

From the Department of Obstetrics and Gynecology, University of Ottawa.

Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.

Reprint requests: Dr. B. Mercer, Department of Obstetrics and Gynecology, Ottawa Civic Hospital, 1053 Carling, Ottawa, Ontario, Canada K1Y 4E9.

Material and methods

A single observer performed 223 postpartum measurements of right heel to first toe within 4 days of delivery. Measurements were performed with a right-angled ruler calibrated in millimeters. Measurements were made without previous knowledge of gestational age. Inclusion requirements were normal pregnancy with neonatal weight appropriate to gestational age[5] and no physical anomaly. Repeat measurements were performed on 20 infants within 2 days of initial assessment.

Two hundred twenty-four ultrasonographic measurements were performed with use of the Siemens and General Electric real-time scanners with a 3.5 mHz lin-

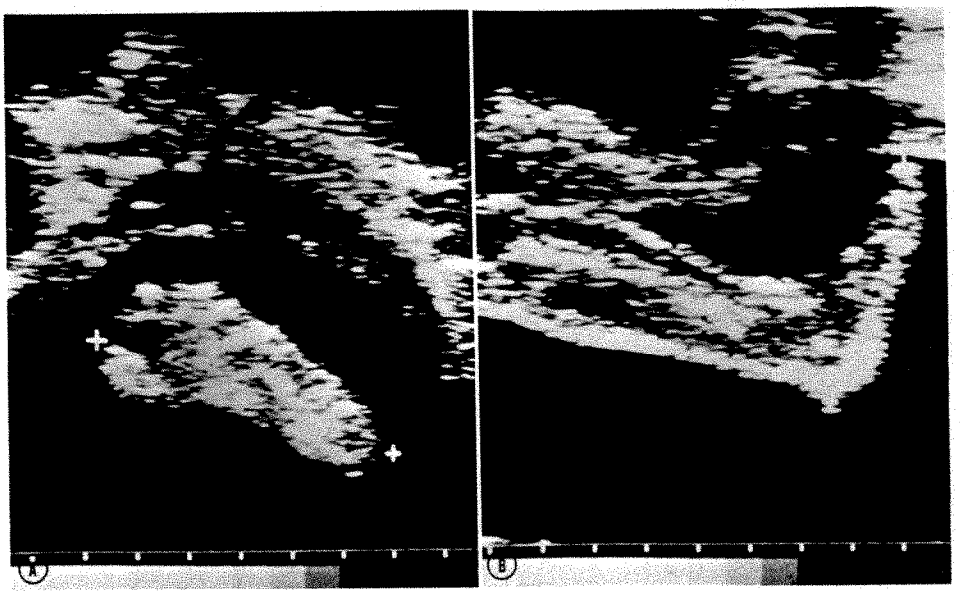


Fig. 1. Ultrasonographic views of fetal foot. *A*, Plantar view with markers at heel and great toe. *B*, Longitudinal view of foot.

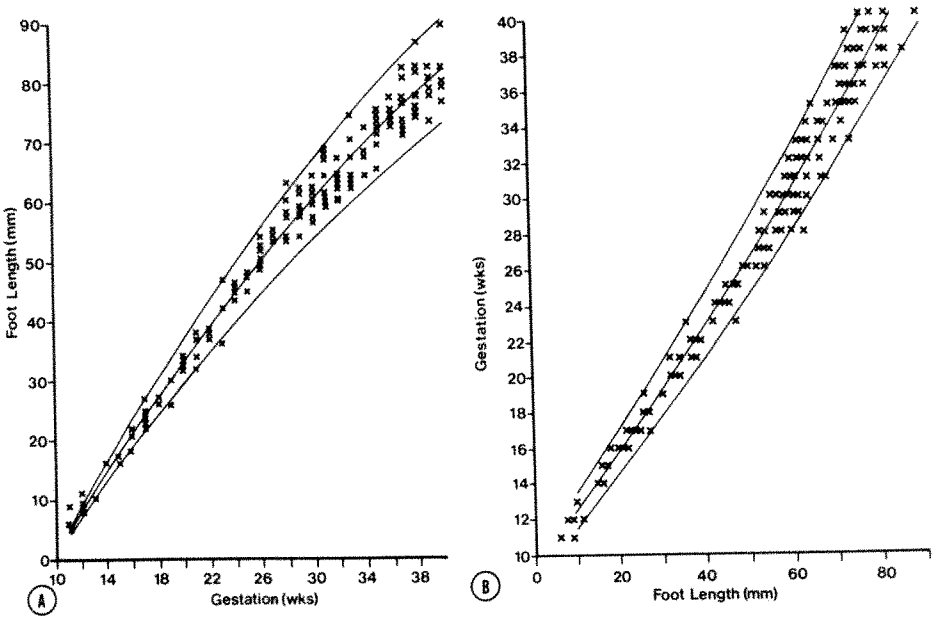


Fig. 2. Scatter plots of ultrasonographic foot length versus gestational age *(A)* and gestational age versus foot length *(B)*. Lines demarcate 5% and 95% confidence intervals as well as predicted value based on curvilinear regressions.

ear-array transducer. Caliper measurements were documented with a Polaroid camera. Both plantar and longitudinal views of the foot were taken when possible (Fig. 1). Requirements for measurement were singleton gestation with secure dates and no identifiable anomaly or intercurrent medical illness. Ultrasonographic measurements were performed on 16 infants of diabetic mothers, eight pairs of twins, and two anencephalic fetuses. Ultrasound correlation to postnatal foot length was assessed with follow-up postpartum measurement of nine infants delivering within 3 days of ultrasonographic assessment.

Mathematical evaluation of the relationship between foot length and gestational age was performed with use of the linear regression model and curvilinear regression models based on exponential, logarithmic, power, and polynomial fits. The variation in foot length at each week of gestation was evaluated by a comparison of

Table I. Comparison of mean postpartum and ultrasonographic foot length with Streeter's pathologic data (1920)

Gestation week	*Streeter's data (mm)*	*Ultrasonographic foot length (mm)*	*Postpartum foot length (mm)*
11	7	8	
12	9	9	
13	11	10	
14	14	16	
15	17	16	
16	20	21	
17	23	24	
18	27	27	
19	31	28	
20	33	33	33
21	35	35	
22	40	38	
23	42	42	
24	45	44	
25	48	47	48
26	50	51	
27	53	54	52
28	55	58	
29	57	57	57
30	59	61	60
31	61	62	60
32	63	63	66
33	65	67	68
34	68	68	71
35	71	71	72
36	74	74	74
37	77	75	78
38	79	78	78
39	81	78	80
40	83	82	81
41			82
42			82
43			84

deviation of observed foot length from predicted values based on the most accurate mathematical model. At each week of gestation 95% confidence intervals were calculated based on ±2 standard deviations from predicted foot length after statistical removal of systemic variation. This process was repeated for gestational age variation as a function of foot length.

Ultrasonographic foot length measurements of the twins, infants of diabetic mothers, and anencephalic fetuses were applied to the predictive curve to determine the reliability of foot length measurements in pregnancies with complicating factors.

Results

Two hundred seven of 223 neonates met our inclusion requirements. The mean foot length at each week of gestation was compared with Streeter's data (Table I). Comparison of 20 serial postpartum measurement pairs revealed similar means and standard deviations, with an average deviation of only 1.7% between measurements (Table II). Paired *t* test evaluation of the two data sets at a 95% confidence level revealed no significant differences, with a beta error of <0.1%.

Requirements for ultrasonographic assessment for the determination of the predictive curve were met by 196 patients; 36 patients did not have adequate documenting photographs and were excluded from further assessment. Measurement initially required up to 7 minutes to identify both poles of the foot. With experience, most feet could be reproducibly measured within 1 minute of scanning time. Difficulty was encountered in patients with oligohydramnios and footling breech presentations, since the foot image was indistinct when compared with the surrounding pelvic soft tissues. Comparison of plantar and longitudinal views revealed similar foot length measurements with insignificant measurement difference between ultrasonographers. Paired *t* test analysis was applied to nine pairs of postpartum measurements following initial ultrasonographic assessment. No significant difference was found at a 95% confidence level with a beta error of <1% (Table II, *B*).

Scatter plots of foot length versus gestational age and subsequently gestation age versus foot length were created (Fig. 2). As with postpartum measurements, mean ultrasonographic foot length at each week of gestation compared very closely to Streeter's data (Table I).

Curvilinear regression with use of the polynomial model demonstrated the best fit to the foot length data. These equations were obtained: (1) Foot length (mm) = $0.02728\ wk^2 + 4.4045\ wk - 36.74$, $R^2 = 0.981$. (2) Gestation (wk) = $7.745 \times 10^{-4}\ mm^2 + 0.3004\ mm + 9.397$, $R^2 = 0.977$.

Residual values from predicted measurements based on equations 1 and 2 were calculated. Systemic variation was noted to increase with gestational age and foot length, respectively (Fig. 3, *A*). This variation was removed by using a transformation in which the residual was expressed as a percentage of the predicted value[6] (Fig. 3, *B*). Standard deviations were calculated for the transformed data. The mean deviation ± 2 SD of the transformed residuals were −0.37 mm ± 11.0% for foot length versus gestational age and 0.07 weeks ± 7.97% for gestational age versus foot length.

Foot length measurements at a gestation of <12 weeks were significantly different from the predicted values with deviations of >30% at 10 and 11 weeks. These values were not included in further evaluation. Based on the regression equations and transformed residual plots, foot length growth and dating curves with 95% confidence interval were derived (Fig. 2, Table III).

Foot length measurements from the infants of diabetic mothers, twin pairs, and anencephalic fetuses all fell within the 95% confidence limits of the predictive curve (Fig. 4).

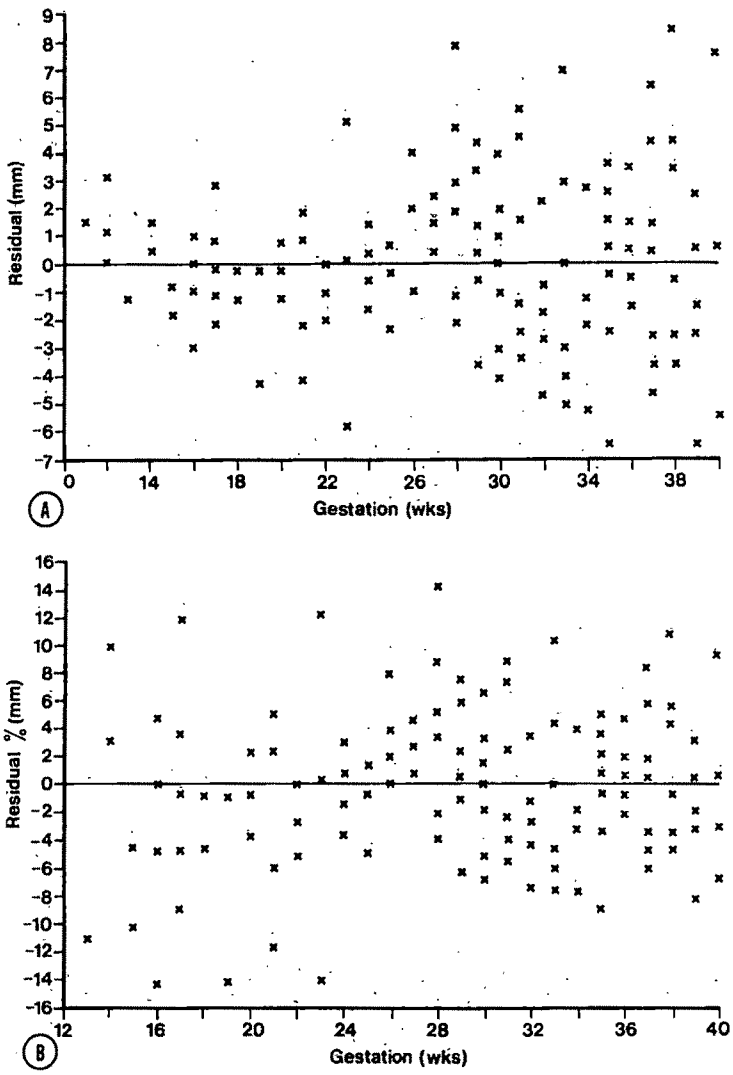


Fig. 3. *A*, Residual plot; *B*, percentage residual plot of fetal foot length versus gestational age.

Table II. Comparison of (1) serial postpartum measurements by single observer within 2 days of initial assessment and (2) postnatal measurements of patients who were delivered within 3 days of delivery

	*Serial postpartum measurements (n = 20)**		*Measurements of patients (n = 9)†*	
	First series (mm)	*Second series (mm)*	*Ultrasonographic*	*Postnatal*
Mean	81.2	81.1	73.0	73.7
Standard deviation	3.30	3.44	8.51	8.04

*Average deviation 1.4 mm (1.72%).
†Average deviation 1.2 mm (1.58%).

Case studies

Case 1. This patient presented at 30 weeks' gestation with a fundal height that was small for dates. Ultrasound assessment demonstrated a biparietal diameter of 78 mm, which was appropriate for gestational age. However, the femur length of 49 mm was appropriate to only 26 weeks' gestation. Foot length measurement of 60 mm confirmed biparietal diameter of gestational age estimation and suggested a presumptive diagnosis of short limb dysplasia. Serial measurements showed progressive delay of long bone growth. The diagnosis of osteogenesis imperfecta was confirmed at birth.

Case 2. The patient was scanned for estimation of gestational age. Biparietal diameter was appropriate for 20 weeks as was the foot length of 33 mm. However, humerus and femur lengths (10 mm) were appropriate

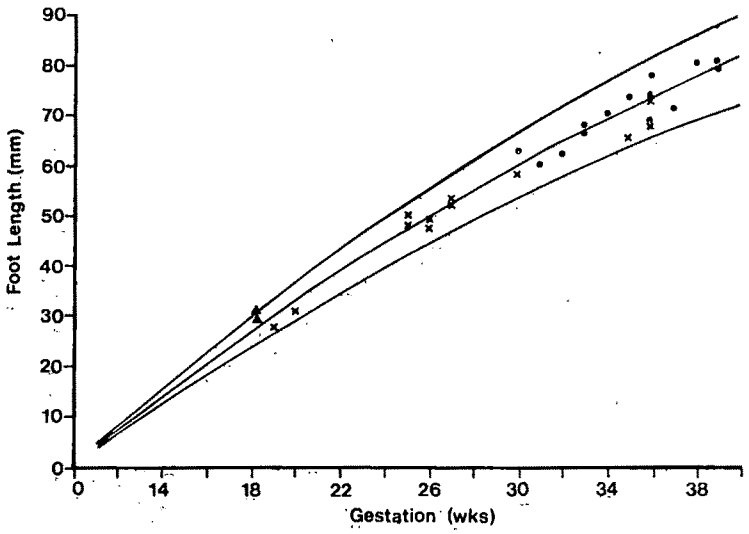


Fig. 4. Fit of twins (×), infants of diabetic mothers (●), and anencephalic fetuses (▲) to regression curve.

Table III. Foot length growth and dating curves based on polynomial regression ±2 SD

Growth curve of foot length				*Dating curve of gestational age*			
Gestational week	*Lower limit (mm)*	*Predicted value (mm)*	*Upper limit (mm)*	*Foot length (mm)*	*Lower limit (wk)*	*Predicted value (wk)*	*Upper limit (wk)*
12	7	8	9	10	11.5	12.5	13.5
13	10	11	12	12	12.1	13.1	14.2
14	13	15	16	14	12.7	13.8	14.9
15	16	18	20	16	13.3	14.4	15.5
16	19	21	23	18	13.9	15.1	16.3
17	22	24	27	20	14.5	15.7	17
18	24	27	30	22	15.1	16.4	17.7
19	27	30	34	24	15.7	17.1	18.4
20	30	33	37	26	16.3	17.7	19.1
21	32	36	40	28	16.9	18.4	19.9
22	35	39	43	30	17.6	19.1	20.6
23	37	42	46	32	18.2	19.8	21.4
24	40	45	50	34	18.9	20.5	22.1
25	42	47	53	36	19.5	21.2	22.9
26	45	50	55	38	20.2	21.9	23.7
27	47	53	58	40	20.8	22.7	24.5
28	49	55	61	42	21.5	23.4	25.2
29	51	58	64	44	22.2	24.1	26
30	54	60	67	46	22.9	24.9	26.8
31	56	62	68	48	23.6	25.6	27.6
32	58	65	72	50	24.3	26.4	28.4
33	60	67	74	52	24.9	27.1	29.3
34	62	69	77	54	25.7	27.9	30.1
35	64	71	79	56	26.4	28.4	30.9
36	66	74	82	58	27.1	29.4	31.8
37	67	76	84	60	27.8	30.2	32.6
38	69	78	86	62	28.5	31	33.5
39	71	80	88	64	29.3	31.8	34.3
40	72	81	90	66	30	32.6	35.2
				68	30.7	33.4	36.1
				70	31.5	34.2	36.9
				72	32.2	35	37.8
				74	33	35.9	38.7
				76	33.8	36.8	39.6
				78	34.5	37.5	40.5
				80	35.3	38.4	41.4
				82	36.1	39.2	42.4
				84	36.9	40.1	43.3
				86	37.7	41	44.2

Table IV. Comparison of 95% confidence intervals for various growth parameters with foot length

Gestational week	*Foot length*	*Femur length*		*Biparietal diameter—Sabbagha et al.*[8]
		Warda et al.[6]	*Jeanty et al.*[7]	
20	±1.6	2.1	2.2	1.7
30	±2.4	3.1	2.2	2.5
40	±3.2	4.0	2.2	–

for only 13 weeks, suggesting a severe short limb dysplasia. Abortion revealed a fetus diagnosed as having osteogenesis imperfecta lethalis.

Comment

Mean foot length from both postpartum and ultrasound groups followed closely Streeter's predictive curve for foot length versus gestational age. There is minimal intraobserver and interobserver variation in both groups of observations.

The polynomial regression curves give the best fit to our ultrasound data with R^2 values of 0.98; 95% confidence intervals based on 2 SD from the predicted values (Table III) are ±1.6 and ±2.4 weeks at 20 and 30 weeks, respectively. These values compare favorably with both biparietal diameter and femur lengths at similar gestations (Table IV). Fetal foot length measurement is a reliable estimator of gestational age between 12 and 40 weeks in normal pregnancies as well as those complicated by diabetes, multiple gestation, anencephaly, and short limb dysplasia.

Ultrasonographic foot length measurement should be undertaken when other parameters do not accurately predict gestational age as well as when abnormality of long bone length or biparietal diameter is suspected. In cases of preterm labor, in which assessment of several growth parameters helps to better approximate gestational age more accurately, the addition of fetal foot length measurement may aid in crucial management decisions.

We respectfully acknowledge Ms. S. Gaudette, Mrs. J. Wotherspoon, and Ms. K. McAuliff for help in the preparation of this paper.

REFERENCES

1. Robinson HP, Fleming JE. A critical evaluation of sonar crown-rump length measurements. Br J Obstet Gynaecol 1975;82:702.
2. Campbell S, Newman GB. Growth of the fetal biparietal diameter during normal pregnancy. J Obstet Gynecol Br Commonw 1968;75:568.
3. O'Brien GD, Queenan JT, Campbell S. Assessment of gestational age in the second trimester by real-time ultrasound measurement of femur length. Am J Obstet Gynecol 1981;139:540.
4. Streeter GL. Weight, sitting height, head size, foot length, and menstrual age of the human embryo. Contrib Embryol Carnegie Inst 1920;11:143.
5. Usher RL, McLean F. Intrauterine growth of live-born caucasian infants born at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J Pediatr 1969;74:901.
6. Warda AH, Deter RL, Rossavik IK, Carpenter RJ, Hadlock FP. Fetal femur length: a critical re-evaluation of the relationship to menstrual age. Obstet Gynecol 1985;66:69.
7. Jeanty P, Rodesch F, Delbeke D, Dumont JE. Estimation of gestational age from measurements of fetal long bones. J Ultrasound Med 1984;3:75.
8. Sabbagha RF, Barton BA. Sonar biparietal diameter. Am J Obstet Gynecol 1976;126:479.

The effect of hydralazine on placental perfusion in the spontaneously hypertensive rat

Jeffrey Lipshitz, M.D., Robert A. Ahokas, Ph.D., and Samuel L. Reynolds, M.S.
Calgary, Alberta, Canada, and Memphis, Tennessee

Intravenous hydralazine was administered to 16 spontaneously hypertensive rats on day 21 of gestation. The radioactive labeled microsphere technique was used to assess the change in organ perfusion produced by the drug. Vascular resistance to most organs was decreased, except to the placentas, stomach, and cecum, where it increased by 43%, 104%, and 44%, respectively. Blood flow to the organs was redistributed, and although it was increased to the lungs, kidneys, liver, and adrenals, it was significantly reduced to the spleen, stomach, placentas, cecum, large intestine, and pancreas. The effect of hydralazine on placental perfusion was opposite to the effect on the uterus (myometrium). Patients with the highest blood pressures tend to have the poorest placental perfusion. Intravenous hydralazine should be used cautiously in these patients. (Am J Obstet Gynecol 1987;156:356-9.)

Key words: Hydralazine, placental perfusion, hypertension

Pregnancy-induced hypertension is associated with increased vascular resistance and decreased uteroplacental blood flow,[1,2] which may in turn lead to perinatal morbidity and mortality. When drugs are used to treat hypertension in pregnancy, it thus becomes important to know the effect of the drug on placental perfusion. The effect of antihypertensive drugs on uteroplacental blood flow has remained controversial because of the lack of appropriate animal models of hypertension during pregnancy and the availability of suitable techniques to measure placental perfusion. The use of uterine artery flow probes to assess placental perfusion in the sheep model appears inappropriate as a drug may produce an increase in myometrial perfusion and a simultaneous decrease in placental perfusion.[3,4]

The genetically spontaneously hypertensive rat is regarded as a good model of human essential hypertension. However, during the latter part of pregnancy, the blood pressure decreases dramatically.[5,6] We have recently demonstrated that carbohydrate supplementation (10% sucrose in the drinking water) could prevent the normal fall in blood pressure during the last week of gestation, thereby producing a model of essential hypertension during pregnancy.[6] By the use of the radioactive labeled microsphere method to assess the distribution of cardiac output and perfusion of various organs, it is possible to distinguish between placental and myometrial perfusion.[4]

The number of drugs available to treat hypertensive disorders in pregnancy has greatly increased during the last few years. The model and technology now exist to test the effect of drugs on placental perfusion and to select drugs for human application on a more rational basis. This report describes the effect of hydralazine on placental perfusion in 16 spontaneously hypertensive rats during pregnancy.

Material and methods

Sixteen spontaneously hypertensive rats with timed pregnancies were obtained from Taconic Farms, Inc., Germantown, New York, and were housed individually in stainless steel wire-bottom cages. The temperature of the room was maintained at 22° C with the lights on from 6:00 AM to 6:00 PM. The animals were fed, as desired, a test diet (Teklad Mills, Madison, Wisconsin) providing the optimum daily amounts of macro- and micro-nutrients for the pregnant rats.[6] They were provided unlimited access to tap water, and from day 5 to day 21, they were given 10% sucrose in tap water to drink.

On day 21 of gestation, the animals were anesthetized with ether, and polyethylene catheters were inserted into the left ventricle of the heart via the right carotid artery and into the femoral artery as previously detailed.[4] The animals were then placed in a plastic restrainer cage and allowed to recover for 3 hours. The femoral arterial catheter was connected to a Statham P23Gb pressure transducer and arterial pressure was recorded on a Gould 2200S recorder. Cardiac output

From the Department of Obstetrics and Gynecology, University of Calgary, and the Department of Obstetrics and Gynecology, University of Tennessee College of Medicine
Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.
Reprint requests: Jeffrey Lipshitz, Division of Obstetrics, University of Calgary at the Foothills Hospital, 1403 29th St. N.W., Calgary, Alberta, Canada T2N 2T9.

Table I. Cardiovascular measurements before and after drug administration

Determination	*Before drug*	*After drug*	*Mean difference*	*p Value*
Mean blood pressure (mm Hg)	116.25 ± 2.95	80.31 ± 3.45	−35.94 ± 3.38	<0.001
Heart rate (bpm)	475.75 ± 5.41	491.20 ± 5.67	16.53 ± 6.99	0.033
Cardiac output (ml/min/100 gm)	36.16 ± 1.37	42.08 ± 2.01	5.93 ± 2.30	0.02
Total peripheral resistance (mm Hg/ml/min/100 gm)	197.02 ± 8.77	119.81 ± 9.20	−77.72 ± 10.88	<0.001

Values are means ± SEM.

and organ blood flow were then determined in the awake rats by the reference sample microsphere technique. A suspension of approximately 100,000 microspheres labeled with either gadolinium 153 or tin 113 (New England Nuclear, Boston, Massachusetts), 15 ± 3 μm in diameter, were sealed in a 15 cm segment of silicone rubber tubing. The microspheres were suspended in physiologic saline solution with Tween-80 (0.01%) added to prevent aggregation. The microsphere-containing catheter was wound around a wooden dowel rod to a height of 1.5 cm, placed in a gamma counting vial, and counted in a Model 1185 gamma well counter (Nuclear-Chicago, Des Plaines, Illinois) at the appropriate photopeak for the isotope used. After counting, the ends of the catheter were clipped and one end was attached to the left ventricular catheter while the other was attached to a syringe containing 0.5 ml of saline solution. The femoral reference blood sample collection was initiated at the rate of 0.5 ml/min, and after verification that it was withdrawing smoothly, the microspheres were flushed into the left ventricle with the 0.5 ml of saline solution during a period of 20 seconds. Collection of the arterial reference sample was continued for 1 minute after the end of the microsphere injection to ensure that all microspheres in transit in the arterial blood were collected. The arterial reference blood sample and syringe washings were transferred to a plastic gamma counting tube. The femoral catheter was flushed with saline solution and reattached to the pressure transducer, and heart rate and arterial blood pressure were recorded again.

The rats were then infused with hydralazine (25 μg/kg/min) via the left ventricle until mean arterial blood pressure decreased by 30 to 40 mm Hg (10 to 45 minutes). Upon completion of the infusion, the alternate set of microspheres was injected and a second arterial reference sample drawn. Each animal was then killed with an overdose of pentobarbital sodium. The entire length of ventricular catheter and silicone rubber tubing was wound around the dowel rod again to a height of 1.5 cm, inserted into the gamma counting tube, and recounted in the gamma well counter. The counts per minute for the calculation of cardiac output was determined as the difference between the counts per minute of the microsphere catheter before injection and the counts per minute of the entire catheter after injection.

After the rat was weighed, the uterus was removed and the fetuses and placentas were carefully detached from the uterine wall. The fetuses and placentas were separated from the amniotic fluid and membranes, gently blotted dry, and weighed separately; the placentas were placed in gamma counting tubes, three to four placentas per tube. The uterus was separated into right and left horns and placed in separate gamma counting tubes. The other organs and tissues were also placed in counting tubes to ensure that the levels of all tissues were kept below 1.5 cm to avoid loss of counting efficiency. The tissues and the arterial reference were counted in the gamma well counter, and the cardiac output and organ distribution of cardiac output were calculated as previously described.[4]

The statistical tests used were the Student t test for between-group comparisons and the paired t test to assess changes within the same groups.

Results

Total peripheral resistance decreased by 39%, resulting in a 36 mm Hg (31%) decrease in mean blood pressure (Table I). Heart rate increased by 4% and cardiac output increased by 16%.

Hydralazine significantly decreased vascular resistance to most organs with the exception of the placentas, stomach, and cecum, where it increased by 43%, 104%, and 44%, respectively (Table II).

Blood flow to the organs was redistributed and, although it increased to some, it was significantly reduced to the spleen (64%), stomach (55%), placentas (44%), cecum (41%), large intestine (39%), and pancreas (33%) (Table III). Hydralazine produced opposite effects on the uterus (myometrum) and placentas.

Rats with the higher blood pressures before drug administration had higher preplacental resistances ($r = 0.64$, $p = 0.004$) and tended to have lower placental blood flows ($r = -0.38$, $p = 0.07$). Resistance and placental perfusion after drug administration correlated significantly with the blood pressure before and after drug administration, and as the drug had a worsening effect on these parameters, the rats with the highest blood pressures before drug administration tended

Table II. Resistance before and after drug administration

Organ	*Resistance (mm Hg/ml/min/100 gm)*			*Change (%)*	*p Value*
	Before drug	*After drug*	*Mean difference*		
Heart	8.9 ± 0.88	4.6 ± 0.45	−4.31 ± 0.83	−48.4	0.0001
Lungs	95.6 ± 14.54	29.4 ± 5.67	−66.19 ± 14.99	−69.2	0.0005
Kidneys	10.2 ± 0.39	6.1 ± 0.46	−4.12 ± 0.70	−40.4	0.0001
Adrenals	15.9 ± 1.13	7.8 ± 0.86	−8.13 ± 1.47	−51.1	0.0001
Liver	2548.1 ± 334.21	1328.9 ± 288.28	−1219.2 ± 351.73	−47.8	0.0035
Spleen	61.1 ± 10.66	168.8 ± 66.66	107.75 ± 68.56	176.4	0.137
Pancreas	80.2 ± 4.80	95.6 ± 15.33	15.44 ± 13.80	19.3	0.281
Stomach	34.8 ± 2.86	70.8 ± 16.11	36.06 ± 15.32	103.6	0.033
Small intestine	15.3 ± 0.98	10.6 ± 1.30	−4.75 ± 1.21	−31.0	0.0014
Cecum	25.9 ± 2.30	37.3 ± 7.12	11.31 ± 5.48	−43.7	0.057
Large intestine	70.1 ± 8.31	75.9 ± 9.73	5.81 ± 11.70	8.3	0.627
Ovaries	10.2 ± 1.06	7.4 ± 0.92	−2.75 ± 0.74	−26.9	0.002
Uterus	108.6 ± 10.05	59.4 ± 7.69	−49.19 ± 9.90	−45.3	0.0002
Placenta	42.7 ± 3.70	61.1 ± 9.31	18.44 ± 7.27	43.2	0.023

Values are means ± SEM.

Table III. Organ blood flow measurements

Organ	*Blood flow (ml/min/100 gm)*			*Change (%)*	*p Value*
	Before drug	*After drug*	*Mean difference*		
Heart	863.85 ± 62.14	1195.61 ± 178.17	331.76 ± 178.93	38.4	0.084
Lungs	97.62 ± 15.87	206.49 ± 19.65	108.87 ± 20.74	111.5	0.0001
Kidneys	686.88 ± 29.40	814.21 ± 40.36	127.33 ± 50.37	18.15	0.023
Adrenals	464.86 ± 29.39	677.82 ± 47.25	212.97 ± 49.87	45.8	0.0007
Liver	3.33 ± 0.36	6.38 ± 1.09	3.05 ± 1.08	91.6	0.013
Spleen	163.45 ± 21.34	59.38 ± 8.73	−104.07 ± 23.25	−63.7	0.0004
Pancreas	90.74 ± 4.71	61.03 ± 5.22	−29.71 ± 4.75	−32.7	0.0001
Stomach	217.44 ± 14.95	97.54 ± 11.01	−119.90 ± 15.06	−55.1	0.0001
Small intestine	480.90 ± 30.43	508.54 ± 39.71	27.64 ± 41.31	5.7	0.51
Cecum	295.96 ± 22.63	175.30 ± 19.73	−120.65 ± 18.56	−40.8	0.0001
Large intestine	115.93 ± 9.88	76.59 ± 7.57	−39.34 ± 11.13	−33.9	0.003
Ovaries	831.10 ± 104.09	766.26 ± 73.37	−64.85 ± 61.36	−7.8	0.31
Uterus	71.83 ± 6.64	112.93 ± 27.77	41.10 ± 22.60	57.2	0.089
Placenta	174.59 ± 9.72	98.22 ± 9.58	−76.37 ± 11.10	−43.7	0.0001

Values are means ± SEM.

Table IV. Correlation coefficients of cardiovascular changes

Variables	*Correlation coefficient (r)*	*p Value*
Pre BP–Pre flow	−0.38	0.07
Pre BP–Pre resistance	0.64	0.004
Pre BP–Post flow	−0.46	0.036
Pre BP–Post resistance	0.52	0.02
Pre flow–Pre resistance	−0.89	<0.001
Post BP–Post flow	−0.50	0.026
Post BP–Post resistance	0.67	0.002
Post flow–Post resistance	−0.89	<0.001

Pre = Before drug administration; Post = after drug administration; BP = blood pressure.

to end up with the lowest placental perfusion after drug administration (Table IV).

Comment

Hydralazine lowers systemic blood pressure through relaxation of vascular smooth muscle. Reflex tachycardia and increased cardiac output occur in response to the decrease in peripheral vascular resistance. Of clinical importance is the differential distribution of cardiac output to the regional vascular beds after administration of hydralazine. Vascular resistance decreased and perfusion increased to organs such as the heart, lungs, kidneys, and liver. However, perfusion decreased to the spleen, pancreas, stomach, cecum, and large intestine. Although vascular resistance to the uterus decreased by 45%, preplacental resistance increased by 43% with a concomitant and highly significant decrease in placental perfusion.

Previous studies,[7, 8] with the use of a hypertensive sheep model with a flow probe on the uterine artery, described a 10% to 15% increase in uterine blood flow after administration of hydralazine. Lunell et al.[9] determined uteroplacental blood flow in 12 hypertensive women before and after the administration of hydralazine. To determine blood flow they injected indium In-99m and used a gamma camera connected to a computer. There was no significant change in uteroplacen-

tal blood flow or in uteroplacental vascular resistance. In both the sheep and human studies described above, placental perfusion could not be distinguished from myometrial blood flow. Unless myometrial and placental perfusion is independently measured, it is not possible to determine the effect of a drug on placental perfusion per se.

Our study confirms that vascular resistance and placental perfusion are correlated with the degree of hypertension (Table IV) and that, after administration of hydralazine, the lowest placental perfusion may occur in the women with the highest blood pressures. In patients with severe pregnancy-induced hypertension, those who received hydralazine had a significantly higher incidence of fetal distress than those who did not receive the drug.[10]

The spontaneously hypertensive rat has not previously been considered as an appropriate model for hypertension during pregnancy as the blood pressure falls progressively during the last week of gestation until it is in the normotensive range by term. The sympathetic nervous system of the spontaneously hypertensive rat (but not the WKY rat) is sensitive to carbohydrate intake.[6] Fasting suppresses sympathetic activity, resulting in a fall in blood pressure,[11] while carbohydrate overfeeding stimulates sympathetic nervous activity, thereby causing an increase in blood pressure.[12] These effects are thought to be mediated through control centers in the ventromedial hypothalamus.[13] We have recently shown that sympathetic nervous stimulation with dietary carboydrate supplementation maintains hypertension until parturition in the pregnant spontaneously hypertensive rat.[6] This appears to be a useful experimental model of essential hypertension in pregnancy in which to evaluate the effects of antihypertensive drugs on placental perfusion.

REFERENCES

1. Lippert TH, Cloeren SE, Fridich R. Assessment of uteroplacental hemodynamics in complicated pregnancy. Int J Gynaecol Obstet 1979;16:274.
2. Suonio S. Maternal placental blood flow in hypertensive pregnancy and the effect of beta-mimetic drugs on it. Dev Pharmacol Ther 1982;4[suppl 1]:99.
3. Katz M, Creasy RK. Uterine blood flow distribution after indomethacin infusion in the pregnant rabbit. AM J OBSTET GYNECOL 1981;1:40:430.
4. Lipshitz J, Ahokas RA, Broyles K, Anderson GD. Effect of hexoprenaline on uteroplacental blood flow in the pregnant rat. AM J OBSTET GYNECOL 1986;154:310.
5. Lorenz RP, Picchio LP, Weisz J, Lloyd T. The relationship between reproductive performance and blood pressure in the spontaneously hypertensive rat. AM J OBSTET GYNECOL 1984;150:519.
6. Ahokas RA, Reynolds SL, Wang Y-F, Anderson GD, Lipshitz J. The effect of carbohydrate overfeeding on blood pressure in the pregnant, spontaneously hypertensive rat. AM J OBSTET GYNECOL 1986;155:1113.
7. Ring G, Krames E, Shnider SM, Wallis KL, Levinson G. Comparison of nitroprusside and hydralazine in hypertensive pregnant ewes. Obstet Gynecol 1977;50:598.
8. Brinkman CR III, Assali NS. Uteroplacental hemodynamic response to antihypertensive drugs in hypertensive pregnant sheep. In: Lindheimer MD, Katz AI, Zuspan FP, eds. Hypertension in pregnancy. New York: John Wiley, 1976:363.
9. Lunell NO, Lewander R, Nylund L, Sarby B, Thornstrom S. Acute effect of dihydralazine on uteroplacental blood flow in hypertension during pregnancy. Gynecol Obstet Invest 1983;16:274.
10. Spinnato JA, Sibai BM, Anderson GD. Fetal distress after hydralazine therapy for severe pregnancy induced hypertension. South Med J 1986;79:559.
11. Young JB, Mullen D, Landsberg L. Caloric restriction lowers blood pressure in the spontaneously hypertensive rat. Metabolism 1978;27:1711.
12. Young JB, Landsberg L. Effect of oral sucrose on blood pressure in the spontaneously hypertensive rat. Metabolism 1981;30:421.
13. Young JB, Landsberg L. Impaired suppression of sympathetic activity during fasting in the gold thioglucose-treated mouse. J Clin Invest 1980;65:1086.

Fetal oxygen extraction: Comparison of the human and sheep

D. Rurak, D.Phil., P. Selke, M.D., M. Fisher, M.D., S. Taylor, A.H.T., and B. Wittmann, M.D.

Vancouver, British Columbia, Canada

To estimate fetal oxygen extraction in the human, the inverse, linear relationship between umbilical arterial Po_2 and oxygen extraction was calculated from measurements made on cord blood samples obtained after vaginal (n = 12) and cesarean (n = 16) delivery and used to predict oxygen extraction in utero. Comparisons with similar data from fetal lambs in utero and during labor and delivery indicate that extraction in the human and sheep fetus is 47% and 27%, respectively, at Pa_{O_2} = 20 mm Hg. For the human fetus to have extraction similar to that in the fetal lamb, vascular Po_2 would have to be substantially higher. This would lead to a higher umbilical venous oxygen content in the human, compensating for the lower umbilical blood flow and yielding a rate of oxygen delivery comparable with that observed in the fetal sheep. These data indicate that there may be significant quantitative differences in the fetal oxygen delivery systems in the human and sheep. (Am J Obstet Gynecol 1987;156:360-6.)

Key words: Fetal oxygen extraction, fetal Po_2, human fetus

Fetal oxygen extraction is the ratio between fetal oxygen consumption and oxygen delivery; it can also be calculated as the difference between umbilical venous (Cv_{O_2}) and arterial (Ca_{O_2}) blood oxygen$_2$ contents divided by umbilical venous oxygen content. In the fetal lamb, oxygen delivery is high, relative to oxygen consumption, and hence extraction is low, normally approximately 30%. When oxygen delivery falls, oxygen consumption can be maintained via an increase in oxygen extraction to approximately 60%.[1] Hence the high oxygen delivery and low extraction serve as an important reserve mechanism to compensate for reductions in fetal oxygen delivery. In the human fetus, umbilical blood flow is reported to be lower than in the fetal sheep, and it has been suggested that this is in part because of the higher hematocrit in the human fetus.[2, 3] However, fetal oxygen consumption appears to be similar in the two species.[4] This could require a higher level of oxygen extraction by the human fetus. Although determination of oxygen extraction in the human fetus in utero would be possible by using percutaneous blood sampling techniques, it is technically easier and ethically more acceptable to estimate oxygen extraction at delivery from measurements of oxygen content in paired cord arterial and venous blood samples.[5] In the present study, measurements of fetal oxygen extraction in the human fetus obtained in this manner are compared to values obtained from chronically instrumented fetal lambs in utero, after exteriorization by uterotomy and during vaginal delivery.

From the Department of Obstetrics and Gynaecology, University of British Columbia, Children's Hospital Research Centre, and Grace Hospital.

Supported by funds from the Medical Research Council of Canada (MA-6459).

Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.

Reprint requests: Dr. D. W. Rurak, Children's Hospital Research Centre, 950 West 28th Ave., Vancouver, British Columbia, Canada V5Z 4H4.

Material and methods

Human studies. Data from 28 human fetuses from 27 women were used in the study. Twelve fetuses were delivered vaginally and 16 by cesarean section. In the one twin pregnancy, twin A was delivered vaginally and twin B was delivered by cesarean section. Details of the pregnancies are given in Table I. In the patients undergoing elective cesarean section, the indications for cesarean section were a previous cesarean section in 10 cases, pregnancy-induced hypertension and intrauterine growth retardation in one case, and a previous infant affected by herpes in one case. The four emergency cesarean sections were performed for (1) delivery of a second breech twin, (2) uterine rupture after a trial of labor in a patient with a previous cesarean section, (3) chorioamnionitis and fetal distress, and (4) failure of labor to progress. All women undergoing cesarean section with epidural anesthesia received 10 L/min of oxygen by face mask, leading to an inspired oxygen concentration of ~40%. Only one infant with acidosis was delivered (pHa = 7.160), and this was in the vaginal delivery group. Postnatal outcome in all infants was normal.

At delivery, paried arterial venous blood samples (0.8 to 3.0 ml) were obtained from a double-clamped length of umbilical cord. The samples were collected in iced, heparinized syringes and stored capped in ice until blood gas analysis was performed, within 10 to 40 minutes of the time of collection.

Table I. Details of human pregnancies in which fetal oxygen extraction was measured

	Vaginal delivery	*Cesarean section*
n	12	16
Length of gestation (wk)	38.9 ± 0.5	37.9 ± 0.6
Birth weight (gm)	3459 ± 165	3228 ± 180
Delivery	8 Spontaneous vaginal delivery	12 Elective
	3 Forceps	4 Emergency
	1 Ventouse	
Anesthesia	6 None	12 Epidural
	5 Epidural	4 General
	1 Pudendal	

Animal studies

Chronically catheterized fetuses in utero. Data from 27 chronically instrumented fetal lambs were used in the present study. The surgical and monitoring procedures have been described previously.[6] Briefly, operation was done at 115 to 130 days' gestation (term ~145 days) with the sheep under halothane anesthesia. Silicone rubber catheters were implanted in a fetal femoral artery, common umbilical vein, lateral tarsal vein, trachea, amniotic cavity, and, in some cases, a maternal femoral artery and jugular or femoral vein. Three groups of fetuses were studied.

NORMOXEMIC FETAL LAMBS (n = 10). These animals were used in a study of the effects of vasopressin on fetal oxygenation.[7] The blood gas data from the control period of these experiments are used as representative values from well-oxygenated fetal lambs.

HYPOXEMIC FETAL LAMBS (n = 12). Twelve fetuses were in this group. Six had arterial P_{O_2} values consistently <15 mm Hg for the entire duration of study, and six had Pa_{O_2} values >15 mm Hg; the mean P_{O_2} level for the entire group was 15.3 ± 0.6 mm Hg. In both groups fetal blood gases and oxygen consumption were measured before, during, and after a 60-minute period of 50% oxygen administration to the ewe. Some of the results from this study have been presented in abstract form.[8] In the present study, the data on Pa_{O_2} and oxygen extraction were used to examine the relationship between the two variables by means of regression analysis.

FETAL LAMBS DURING LABOR AND DELIVERY (n = 5). These animals were monitored continuously before and during spontaneous labor and vaginal delivery. The total monitoring time during labor ranged from 11 to 17.5 hours (mean = 13.9 ± 1.3). Measurements of blood gases, umbilical blood flow, and oxygen consumption were made every 2 hours until the last 2 to 3 hours of labor when the average sampling frequency was approximately 15 minutes. All of the fetuses were born alive, but one severely acidemic lamb died after several hours. Three sets of blood gas and oxygen extraction data from these preparations are presented in this report. Prelabor samples were collected the day before the onset of labor; early labor samples were obtained when increased uterine activity was first noted (13.9 ± 1.3 hours before delivery; and late labor samples were collected 4.2 ± 1.0 minutes before delivery, when the fetal head was in the vagina.

Exteriorized fetal lambs. Four exteriorized fetuses were studied at 130 to 140 days' gestation. They were used to compare umbilical blood flow estimates made with an electromagnetic flowmeter on the common umbilical artery with the steady-state antipyrine diffusion technique. These results were reported previously[6]; however, the blood gas data from these animals were not reported. The fetuses were delivered with the ewes under pentobarbital sodium anesthesia with care taken to minimize disturbance of the umbilical circulation and were kept adjacent to the ewe on a heated table. Observations were made for a period of 3 to 5 hours.

Measurement techniques. Blood gas values and pH were measured with an 11 micro 13 blood gas analyzer (Instrumentation Laboratory, Lexington, Massachussetts). The measurements were made at 39° C for the sheep samples and at 37° C for the human samples. Blood oxygen content was measured with a $LexO_2$ Con-K oxygen analyzer (Lexington Instruments, Waltham, Massachussetts). Fetal oxygen extraction was calculated as umbilical venous minus arterial oxygen content divided by umbilical venous content.

Differences in the measured variables among the various study groups were tested for statistical significance by use of the unpaired *t* test. Linear regression analysis, by the least-squares method, and significance tests for differences in slopes and y intercepts between different regression equations were done as described by Zar.[9] Values are presented as mean ± SE.

Results

Table II gives the mean values for umbilical arterial and venous blood gases and for fetal oxygen extraction for five groups of results: human fetuses delivered by cesarean section and vaginally, fetal lambs in utero, exteriorized fetal lambs, and fetal lambs during labor and delivery, with the latter group composed of prelabor, early labor, and late labor results. For the human

Table II. Umbilical cord arterial and venous blood gas values, base excess, packed cell volume, oxygen content, venous-arterial oxygen difference, and oxygen extraction in humans and sheep

Group	*n*	*Site*	*Po_2 (mm Hg)*	*Pco_2 (mm Hg)*	*pHa*
Human C/S	16	Artery	16.2 ± 1.3	51.5 ± 1.1	7.324 ± 0.012
		Vein	29.7 ± 2.3	40.7 ± 4.0	7.372 ± 0.011
Human V/D	12	Artery	16.3 ± 1.5	48.1 ± 1.5	7.277 ± 0.014*
		Vein	27.9 ± 1.0	36.8 ± 0.6	7.344 ± 0.011*
Sheep I/U	10	Artery	20.0 ± 1.0†	49.2 ± 0.8	7.414 ± 0.005†
		Vein	29.9 ± 1.9	43.6 ± 0.9	7.432 ± 0.007†
Sheep ext.	4	Artery	21.1 ± 1.8	52.8 ± 2.2	7.275 ± 0.087‡
		Vein	32.3 ± 3.2	44.2 ± 2.5	7.313 ± 0.081‡
Sheep V/D	5				
Before		Artery	17.8 ± 1.5	48.2 ± 1.5	7.398 ± 0.039
		Vein	25.8 ± 2.0	42.6 ± 1.2	7.407 ± 0.050
Early		Artery	15.6 ± 1.5	46.7 ± 1.9	7.376 ± 0.052
		Vein	26.2 ± 3.0	40.4 ± 1.6	7.408 ± 0.058
Late		Artery	13.0 ± 0.8‡	53.1 ± 4.2	7.250 ± 0.086‡
		Vein	25.4 ± 2.9	43.9 ± 2.4	7.290 ± 0.077‡

Human C/S, Humans delivered by cesarean section; human V/D, humans delivered vaginally; sheep I/U, fetal lambs in utero; sheep ext., exteriorized fetal lambs; sheep V/D, fetal lambs during labor and vaginal delivery.

*$p < 0.05$ for difference from "Human C/S" value.

†$p < 0.05$ for difference from pooled "Human" values.

‡$p < 0.05$ for difference from "Sheep I/U" value.

Table III. Linear regression analysis of relationship between fetal oxygen extraction and arterial Po_2 and pH in human fetuses at delivery, fetal lambs in utero, and fetal lambs during labor and delivery

	n	*a*	*b_1*	*p*	*b_2*	*p*
Humans*	28	91.0†	−2.24 ± 0.38	<0.001	—	—
Sheep in utero*	49	57.3	−1.47 ± 0.18	<0.001	—	—
Sheep in labor‡	50	91.5†	−3.27 ± 0.49†	<0.001	53.2 ± 5.7	<.00

*Equation: oxygen extraction = $a + b_1 (Pa_{O_2})$.

†$p < 0.001$ for difference from sheep in utero value.

‡Equation: oxygen extraction = $a + b_1 (Pa_{O_2})$.

data, umbilical arterial and venous pH and base excess were significantly lower after vaginal delivery than after cesarean section, but there were no other differences between the two sets of human results. In both groups, there was a wide variation in vascular Po_2 and oxygen content; for Pa_{O_2}, the values ranged from 5.9 to 24.0 mm Hg for the vaginal delivery group and from 7.7 to 26.1 mm Hg for the cesarean section group. A similar wide range was observed for Ca_{O_2} and for umbilical venous oxygen levels.

For comparison with the sheep data, the human vaginal delivery and cesarean section results were pooled. Pa_{O_2}, pH, base excess, and umbilical arterial oxygen content in the human were lower compared with the values obtained from normoxemic fetal lambs in utero. Pco_2 and umbilical venous Po_2 and oxygen contents were not different between the human and antenatal sheep data, whereas hematocrit, the umbilical venous-arterial oxygen difference ($Cv_{O_2} - Ca_{O_2}$), and oxygen extraction were significantly higher in the human fetus. However, with exteriorization of the fetal lamb and during labor and delivery there was a deterioration in fetal blood gas status. With the exteriorized lambs, pH and base excess were significantly lower than with the fetuses in utero, whereas during labor there were progressive and significant falls in Pa_{O_2}, pH, base excess, Ca_{O_2}, and Cv_{O_2}. In both the exteriorized fetuses and the lambs at delivery, the umbilical venous-arterial oxygen difference and oxygen extraction were significantly greater than for the fetuses in utero but were not significantly different from the pooled human value.

Fig. 1 illustrates the relationship between Pa_{O_2} and fetal oxygen extraction in hypoxemic fetal lambs before, during, and after administration of a 50% oxygen mixture to the mother. There was a significant, linear relationship between these two variables ($r = 0.746$; $p < 0.001$). Similar plots are given in Figs. 2 and 3 for the human data and for the results obtained from fetal lambs in labor. For both sets of data, there was a significant relationship between Pa_{O_2} and oxygen extrac-

Base excess mEq/L	*Packed cell volume (%)*	*Oxygen content vol/100 ml*	*Venous-arterial oxygen difference vol/100 ml*	*Oxygen extraction (%)*
−0.8 ± 0.5	45.5 ± 1.4	5.6 ± 0.6 12.5 ± 0.9	6.8 ± 0.6	55.1 ± 3.0
−5.2 ± 0.7*	46.9 ± 1.4	5.9 ± 0.8 12.1 ± 0.7	6.1 ± 0.7	51.9 ± 5.1
+5.5 ± 0.6†	37.3 ± 0.8†	7.9 ± 0.6† 11.3 ± 0.5	3.4 ± 0.3†	31.0 ± 3.0†
−2.6 ± 5.8‡	42.9 ± 2.5	7.7 ± 1.2 12.6 ± 1.3	4.9 ± 0.2‡	40.5 ± 4.3‡
+4.1 ± 3.0	39.7 ± 3.0	7.7 ± 0.6 11.1 ± 0.8	3.3 ± 0.6	29.6 ± 4.5
+1.5 ± 3.1	39.8 ± 3.3	5.6 ± 2.5 10.5 ± 0.9	4.8 ± 0.8	46.2 ± 6.6‡
−4.3 ± 4.6‡	36.8 ± 3.7	3.6 ± 0.7‡ 8.8 ± 1.0‡	5.2 ± 0.9‡	59.2 ± 7.8‡

tion; r values for the human and sheep were 0.768 and 0.867. For the fetal lambs in labor there was also a significant relationship between arterial pH and oxygen extraction, and the marked variability in oxygen extraction at Pa_{O_2} between 8 and 16 mm Hg illustrated in Fig. 3 is in large part the result of variations in pHa (6.966 to 7.439) in the different fetuses at the end of labor.

Table III lists the regression coefficients and y intercept values for the regression equations calculated for the human and sheep data illustrated in Figs. 1 to 3. In the comparison of the pooled human results with the data from fetal lambs in utero, there was no significant difference in the respective regression coefficients, whereas the y-intercept value for the human (91.0) was significantly greater ($p < 0.001$) than the value in the sheep (57.3). For the fetal lambs in labor, the y-intercept value for $Po_2 = 0$, pH = 7.41 was 91.5, which was not different from the human value but was significantly higher than the value obtained from fetal lambs in utero. Moreover, the regression coefficient for Po_2 in the sheep labor group (−3.27 ± 0.49) was significantly higher than the coefficient from lambs in utero (−1.47 ± 0.18). The calculated regression equations for the three sets of data were used to predict fetal oxygen extraction at $Pa_{O_2} = 20$, pHa = 7.41. The calculated value for the pooled human data was 47.0%. This was significantly higher ($p < 0.001$) than the extractions of 27.3% and 25.8% calculated for the antenatal and intrapartum sheep data.

Comment

The results of this study indicate that oxygen extraction in the human fetus is substantially higher than in the fetal lamb, at least if vascular Po_2 is similar in the two species. The comparison between the two species was carried out by examining the relationship between umbilical arterial Po_2 and oxygen extraction and using the calculated regression equation to predict extraction in utero for $Po_2 = 20$ mm Hg. The linear, inverse relationship between the two variables does not indicate a direct causal relationship between the two variables. Rather it indicates that oxygen extraction and arterial Po_2 are altered in opposite directions by changes in either oxygen delivery or fetal oxygen consumption. For example, for a constant level of oxygen consumption, an increase in delivery would result in a fall in extraction and a rise in Po_2.[7] Conversely, a fall in delivery would lead to a rise in extraction and a decline in Po_2.[1] Similar reciprocal changes in the two variables would occur with alterations in fetal oxygen consumption when oxygen delivery is constant.[6, 10] Mathematically, an inverse, linear relationship between oxygen extraction and Po_2 is to be expected for the following reasons. First, as stated in the introduction,

$$\text{Oxygen extraction} = (Cv_{O_2} - Ca_{O_2})/Cv_{O_2}.$$

This can be rearranged to the form,

$$\text{Oxygen extraction} = 1 - Ca_{O_2}/Cv_{O_2}$$

Second, with both the human and the sheep data, Cv_{O_2} and Ca_{O_2} are significantly correlated. For example, with the human data, $Cv_{O_2} = 7.22 + 0.88\,Ca_{O_2}$ ($p < 0.001$). Substituting this into the equation for extraction yields

$$\text{Oxygen extraction} = \frac{1 - 0.017\,Ca_{O_2}}{1 - 0.12\,Ca_{O_2}}$$

This equation predicts an inverse relationship between the two variables, but it is not linear; instead, the negative slope decreases with increasing values of Ca_{O_2}. However, over the normal range of Ca_{O_2} ($\leq \sim 8$ vol/100 ml), the relationship closely approximates a

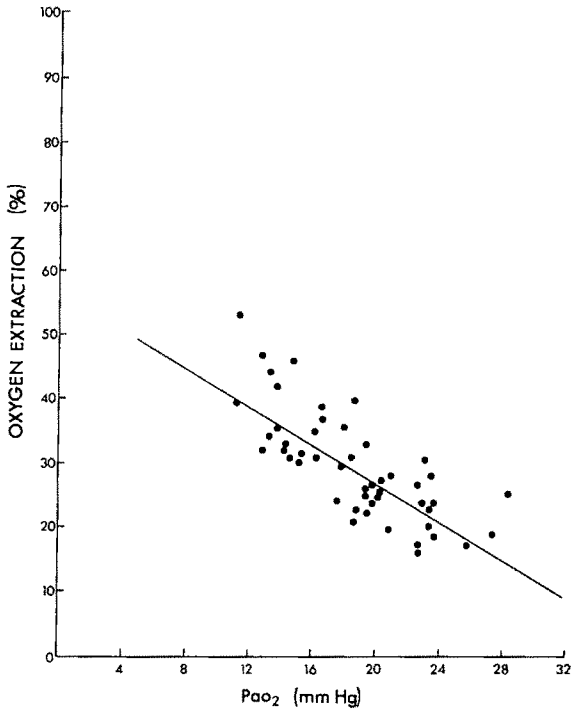


Fig. 1. Relationship between umbilical arterial P_{O_2} (Pa_{O_2}) and oxygen extraction in 13 fetal lambs before, during, and after administration of 50% oxygen to the mother. The line was fitted by regression analysis and conforms to the equation: oxygen extraction = 57.3 − 1.47 (Pa_{O_2}).

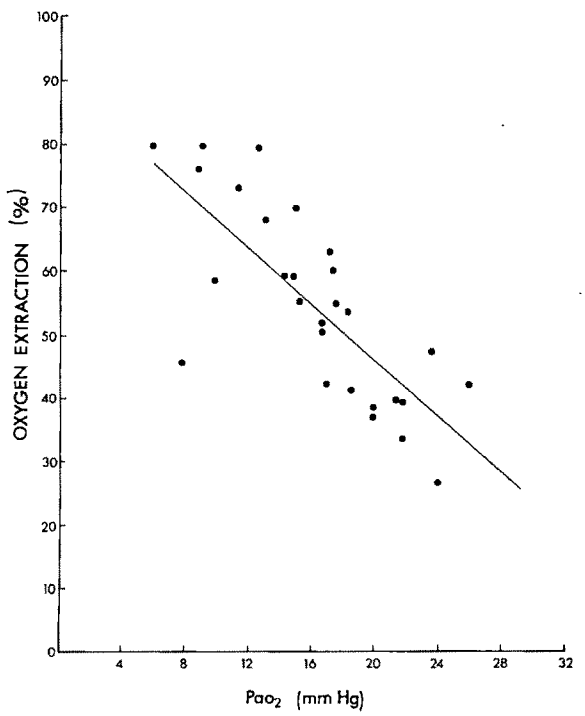


Fig. 2. Relationship between umbilical arterial P_{O_2} and oxygen extraction estimated in cord blood samples collected from 28 human fetuses after vaginal delivery (n = 12) or cesarean section (n = 16). The line was fitted by regression analysis and conforms to the equation: oxygen extraction = 91.0 − 2.24 (Pa_{O_2}).

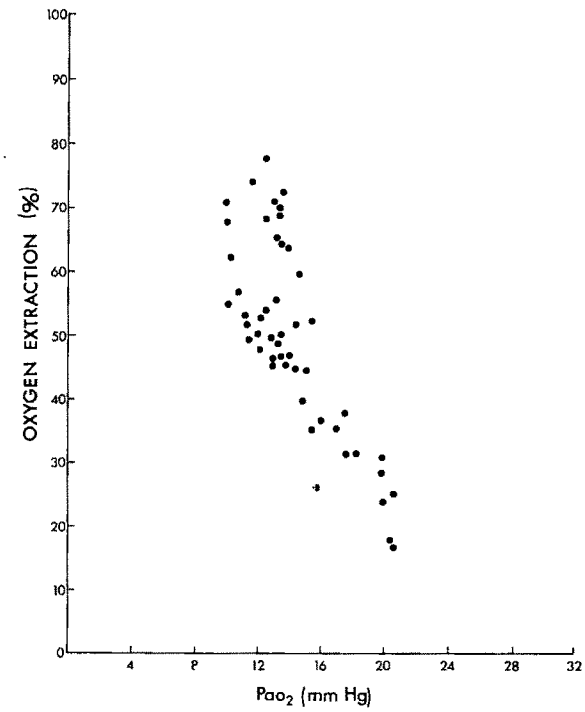


Fig. 3. Relationship between umbilical arterial P_{O_2} and oxygen extraction in five fetal lambs during labor and delivery. The relationship is best described by the equation: oxygen extraction = 91.5 − 3.27 (Pa_{O_2}) − 53.2(pHa).

straight line (extraction = 0.93 − 0.071 Ca_{O_2}, r = 0.993). Finally, over the physiologic range of fetal P_{O_2}, there is a linear relationship between P_{O_2} and oxygen saturation[11]; hence one would expect an inverse, linear relationship between extraction and Pa_{O_2}, as was found in the present study.

There are clear differences in the conditions under which the measurements of human fetal oxygen extraction were obtained compared with those under which the antenatal data were collected from sheep. However, analysis of the information obtained from sheep during labor and delivery resulted in a predicted fetal oxygen extraction similar to that calculated from the data collected from lambs in utero (25.8% and 27.3%, respectively, at P_{O_2} = 20 mm Hg). Hence the use of the regression analysis to predict human fetal oxygen extraction in utero from cord blood data obtained at delivery appears to be valid.

There do not appear to be any published studies on human fetal oxygen extraction, although some reports give data on umbilical venous and arterial oxygen saturation or content that can be used to calculate extraction. Ramanathan et al.[12] studied women undergoing elective cesarean section who were divided into four groups on the basis of the oxygen concentration administered, which ranged from 21% to 100%. From their data, a regression equation for the relationship between umbilical arterial P_{O_2} and fetal oxygen ex-

traction can be calculated. The regression coefficient (−2.64) and y-intercept value (100.1) were similar to values obtained in the present study. In addition, the calculated extractions at P_{O_2} = 20 mm Hg are similar in the two studies (47.5 versus 47.0).

Aside from the higher oxygen extraction noted in the human fetus, there were also a lower P_{O_2} and pH compared with those of fetal lambs in utero. These differences are likely due in part to the delivery process, inasmuch as similar changes were observed during labor in sheep. During vaginal delivery, fetal oxygen delivery could be impaired by reductions in uterine and umbilical blood flow during uterine contractions, whereas with cesarean section there could be impairment of maternal and fetal-placental blood flows by uterine manipulation or maternal aortic and caval compression, in turn leading to reductions in fetal oxygenation[13]. The umbilical venous-arterial difference in oxygen content is also higher in the human fetuses than in the fetal lambs in utero. Although this could be a consequence of a reduced umbilical blood flow during delivery, a venous-arterial oxygen difference of 6 to 7 vol/100 ml is also consistent with the reported values for umbilical blood flow (120 ml/min · kg)[2] and oxygen consumption (7.6 ml/min · kg)[4] in the human fetus.

Another finding in the present study is the wide variation in umbilical arterial and venous P_{O_2} with both vaginal delivery and cesarean section. In vaginal delivery, the variation may relate to differences in the duration or severity of labor. With cesarean section, it may be due more to variations in the uterine incision-to-delivery time interval, inasmuch as Datta et al.[13] reported a deterioration in fetal blood status during cesarean section when this interval was increased beyond 3 minutes. In any event, the variation in cord blood P_{O_2} makes it difficult to predict normal vascular oxygen tension in the human fetus in utero. This is an important requirement in the present study, because the conclusion that oxygen extraction is higher in the human fetus is based in part on the assumption that human fetal umbilical arterial P_{O_2} is similar to that observed in the fetal lamb (~20 mm Hg), yielding a value for extraction of ~47%. For extraction in the human to be 30%, Pa_{O_2} would have to be ~27 mm Hg. In physiologic terms, this means that for the human fetus to have a level of oxygen extraction comparable to that of the fetal lamb, umbilical venous P_{O_2} and oxygen saturation would have to be high enough to compensate for the lower rate of umbilical blood flow in the human and hence yield a value for oxygen delivery similar to that in the sheep fetus. To achieve this, umbilical venous blood would have to be nearly fully saturated with a P_{O_2} of 50 to 55 mm Hg. This is higher than the accepted normal range for the fetal lamb but is similar to values reported for the fetal foal and cow.[14] Recently, Nicolaides et al.[15] reported human umbilical venous blood gas values measured in samples obtained by percutaneous fetal blood sampling in second-trimester pregnancies. The mean P_{O_2} was ~55 mm Hg. It can be calculated from an assumed umbilical venous-arterial oxygen difference of 6 to 7 vol/ml that umbilical arterial P_{O_2} would be ~30 mm Hg, which compares favorably with the predicted value of 27 mm Hg in the present study.

If the high vascular P_{O_2} reported for the human fetus in the second trimester[15] is also present in the third trimester, the human fetus undergoes a much more profound deterioration in blood gas status during the delivery process than has been found for the fetal lamb in this and previous studies.[16] Moreover, it could also mean that oxygen delivery and extraction in the human fetus are similar to the levels observed in the fetal lamb. However, the results would be achieved in different ways in the two species. In the lamb, delivery is high largely because cardiac output and umbilical blood flow are high. In contrast, in the human, a comparable rate of oxygen delivery would be achieved in part by the higher hemoglobin concentration and in part by a higher umbilical venous P_{O_2} and oxygen saturation, with the latter differences suggesting a more efficient system for placental oxygen transfer than in the sheep. These differences, and others,[3] indicate significant quantitative differences in the systems for fetal oxygen delivery in the human and sheep.

REFERENCES

1. Edelstone DI. Fetal compensatory responses to reduced oxygen delivery. Semin Perinatol 1984;8:184-91.
2. Erskine RLA, Ritchie JWK. Quantitative measurement of fetal blood flow using Doppler ultrasound. Br J Obstet Gynaecol 1985;92:600-4.
3. Huikeshoven FJ, Hope ID, Power GG, Gilbert RD, Longo LD. A comparison of sheep and human fetal oxygen delivery systems using a mathematical model. Am J Obstet Gynecol 1985;151:449-55.
4. Bonds DR, Haggerdahl M, Gabbe SG. Estimation of human fetal-placental unit oxygen consumption by application of the Bohr Principle [Abstract]. In: Proceedings of the thirtieth annual meeting of the Society for Gynecologic Investigation, Washington, D. C., March 17-20, 1983. Washington DC: Society for Gynecologic Investigation, 1983:3.
5. Daffos F, Forestier F, Pavlosvsky MC. Fetal blood sampling during the third trimester of pregnancy. Br J Obstet Gynaecol 1984;91:118-21.
6. Rurak DW, Gruber NC. Increased oxygen consumption associated with breathing activity in fetal lambs. J Appl Physiol 1983;54:701-7.
7. Rurak DW, Gruber NC. The effect of vasopressin on fetal oxygenation in sheep. Can J Physiol Pharmacol 1984; 62:27-30.
8. Rurak D, Taylor S. Maternal oxygen administration and fetal oxygen consumption [Abstract]. In: Proceedings of twelfth annual meeting of the Society for the Study of Fetal Physiology, Haifa, Israel, July 8-11, 1985. Haifa, Israel: Society for the Study of Fetal Physiology, 1985:24.
9. Zar JH. Biostatistical analysis. Englewood Cliffs, New Jersey: Prentice-Hall, 1984:292.

10. Rurak DW, Gruber NC. The effect of neuromuscular blockade on oxygen consumption and blood gases in the fetal lamb. Am J Obstet Gynecol 1983;145:258-62.
11. Nathanielsz PW, Yu HK, Cabalum TC. The effect of abolition of fetal movement on the incidence of tonic myometrial contractures in the pregnant ewe at 114 to 134 days' gestation. Am J Obstet Gynecol 1982;144:614-18.
12. Ramanathan S, Gandhi S, Arismendy AAS, Chalon J, Turndorf H. Oxygen transfer from mother to fetus during cesarean section under epidural anesthesia. Anesth Analg 1982;61:576-81.
13. Datta S, Ostheimer GW, Weiss JB, Brown WU, Alper MH. Neonatal effect of prolonged anesthetic induction for cesarean section. Obstet Gynecol 1981;58:331-5.
14. Comline RS, Silver M. Placental transfer of blood gases. Br Med Bull 1974;31:25-31.
15. Nicolaides KH, Rodeck CH, Soothill PW, Campbell S. Ultrasound-guided sampling of umbilical cord and placental blood to assess fetal wellbeing. Lancet 1986;1:1065-7.
16. Comline RS, Silver M. The composition of foetal and maternal blood during parturition in the ewe. J Physiol 1972;222:233-56.

Endometrial carcinoma occurring in patients under the age of 45 years

J. D. Jeffery, M.D., R. Taylor, M.D., D. I. Robertson, M.D., and G. C. E. Stuart, M.D.
Calgary, Alberta, Canada

Endometrial adenocarcinoma is frequently unsuspected in women under the age of 45 years by the gynecologist or the pathologist, but it does occur. Twenty-seven cases of endometrial adenocarcinoma in this age group, from 1980 to 1985, were reviewed clinically and pathologically. Five cases were excluded by histologic examination. Obesity and abnormal vaginal bleeding were shown to be risk factors. Endometrial screening is to be encouraged. This cancer may arise de novo rather than from a premalignant precursor. Implications of this neoplasm for the premenopausal woman are considered. (Am J Obstet Gynecol 1987;156:366-70.)

Key words: Carcinoma, endometrium, younger age group

Endometrial adenocarcinoma is classically a disease of the older, post-menopausal woman. Obesity, impaired carbohydrate metabolism, nulliparity, late onset of menopause, hypertension, anovulatory menstrual cycles, and exogenous estrogen administration have been documented as risk factors for the development of this neoplasm in the population at risk. These same risk factors may not apply to a younger population, yet 2% to 3% of cases of endometrial adenocarcinoma occur in women under the age of 45 years.[1] Gallup and Stock[2] identified 14.4% of their cases to have been diagnosed at age 40 years or less. Endometrial cancer is frequently unsuspected clinically or pathologically in women under the age of 45 years. This tumor does exist and may arise de novo in some cases in the presence of otherwise normal-appearing, functioning endometrium. The presence of obesity and abnormal vaginal bleeding should encourage a greater practice of endometrial sampling. The purpose of this retrospective, clinicopathologic study is to evaluate the risk factors in patients among this premenopausal subgroup and to describe their disease histologically.

From the Departments of Obstetrics and Gynaecology and Pathology, University of Calgary.
Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.
Reprint requests: Dr. G. C. E. Stuart, Director of Gynaecology, Tom Baker Cancer Centre, 1331-29 St. NW, Calgary, Alberta, Canada T2N 4N2.

Material and methods

Twenty-seven patients diagnosed from 1980 to 1985 as having endometrial adenocarcinoma were accessioned through the Cancer Registry at the Tom Baker Cancer Centre, Calgary, Alberta, Canada. Women under the age of 45 years were selected to obtain a premenopausal study group for comparison with current literature. Clinical data were obtained by chart review of the time of diagnosis and by correspondence, as necessary, with the primary care physicians. Ideal body weight was assumed to be 100 pounds for a height of 60 inches, with an allowed increase of 5 pounds for each inch over a height of 60 inches. Two of the authors (R. T., D. I. R.) reviewed the original histologic material of all patients, as well as recuts on selected blocks of tissue as necessary. Statistical analysis was done by the χ^2 method.

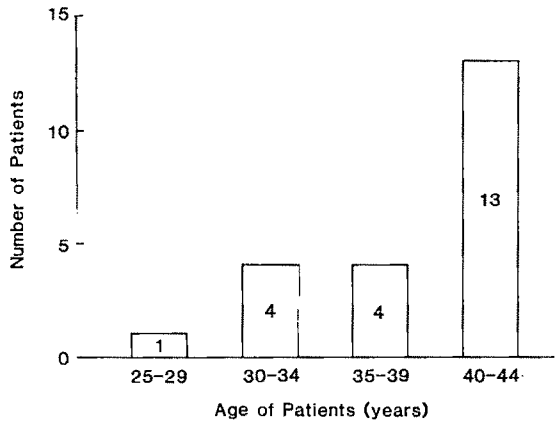


Fig. 1. Distribution of patients' ages.

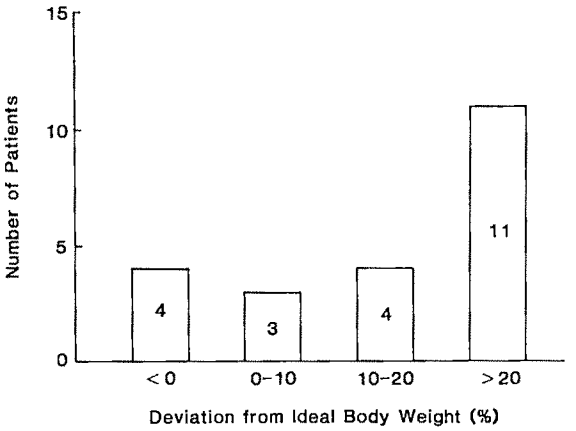


Fig. 2. Distribution of relative weights.

Table I. Menstrual pattern at time of diagnosis

Bleeding pattern		*No. of patients*
Cycle	*Flow*	
Irregular	Heavy	10
Irregular	Light	2
Irregular	Normal	1
Regular	Heavy	8
Regular	Normal	1
		22

Table II. Surgical management of endometrial adenocarcinoma

Procedure	*No. of patients*
Total hysterectomy with bilateral salpingo-oophorectomy	17
Total hysterectomy only	4
Cervical dilatation and uterine curettage (lost to follow-up)	1

Results

Five patients were excluded after histologic review, with an error in coding for uterine malignancy other than endometrial adenocarcinoma or a disagreement with the histologic diagnosis of endometrial adenocarcinoma. The remaining 22 patients under the age of 45 years represent 5% of cases of endometrial adenocarcinoma documented in Southern Alberta from 1980 through 1985. The mean age of these 22 patients was 39.0 years (range 26.6 to 44.9 years, see Fig. 1). The mean age of menarche, identified in nine patients, was 12.8 years (range 9 to 15 years); nine patients were nulligravid, and 13 (59%) were parous.

Eleven patients (50%) were more than 20% above their ideal body weight (Fig. 2). Eight (36.4%) of the twenty-two women were smokers. The mean arterial blood pressure of all patients was 91.4 ± 12.7 mm Hg. Before diagnosis, only two patients were receiving antihypertensive medications; only one patient had been recognized as having diabetes mellitus, type II. Two patients had exposure to clomiphene citrate as an ovulation induction agent, within 12 months before diagnosis of disease. It was not possible from chart review to draw any meaningful conclusions regarding incidence of oral contraceptive pill use among the study patients. One of the 22 study patients had a bilateral salpingo-oophorectomy performed 10 years before diagnosis, because of bilateral mucinous cystadenomas, but this patient had been maintained on a regimen of cyclic conjugated estrogens during the interval.

The most common physical findings documented by the assessment of the admitting physician at the time of diagnosis were clinically increased uterine size (nine of 22 patients) (40.9%) and obesity (eight of 22 patients) (36.4%). All patients but one had a reported abnormality of the usual menstrual pattern (see Table I). Heavy or irregular menses was defined as an increase or change by more than 2 days from the regular menstrual pattern.

Five patients (22.7%) failed to have carcinoma diagnosed before hysterectomy. The remaining 17 patients each had carcinoma diagnosed on prehysterectomy uterine curettings. One patient has been lost to follow-up after the diagnosis was made by fractional curettage. Surigcal management is as listed in Table II. Surgical therapy was performed at various referring institutions before review at the Cancer Centre. Three patients have received postoperative external beam pelvic radiation, followed by vaginal vault radiation. Two of these cases were for histologic evidence of endocervical involvement and the third patient had Stage IB, grade 1 disease with full-thickness myometrial invasion. One patient has received chemotherapy in the form of Adriamycin, cyclophosphamide, and progestogens. She represents the only patient to die in the present series and had an associated second primary

Table III. Stage and grade (according to International Federation of Gynecology and Obstetrics) compared with myometrial invasion

Clinical stage and grade	*Tumor confined to endometrium*	*Superficial (<1/3) myometrial invasion*	*Deep (>1/3) myometrial invasion*	*Row total*
IA, G1	6	1	0	7
IA, G2	0	1	0	1
IB, G1	4	3	1	8
IB, G2	1	0	1	2
II, G1	2	1	0	3
Column total	13	6	2	21

Table IV. Associated gynecologic pathologic conditions

Associated gynecologic pathologic condition	*Clinical stage and grade of endometrial adenocarcinoma*
Bilateral endometrioid adenocarcinoma of ovaries	IA, G1
Chronic pelvic inflammatory disease–old, healed salpingitis	IB, G1
Focal adenomatoid tumor within uterine wall	IB, G2
Bilateral mature cystic teratomas (dermoid cysts)	II, G1

Table V. Precursor lesions in endometrial adenocarcinoma

Lesion	*Patients*	
	No.	*%*
Adenomatous hyperplasia with or without atypia		
Present	15	68.2
Absent	7	31.8
Evidence of functioning background endometrium		
Present	15	68.2
Absent	7	31.8

malignancy of Stage III endometrioid adenocarcinoma of the ovary. A fourth patient has received external beam pelvic radiation followed by administration of Alkeran, tamoxifen, and dexamethasone as postoperative adjuvant therapy for clinical Stage IB–surgical Stage IV disease. All adjunctive therapy was given at the Cancer Centre. All patients but one are alive and well without evidence of recurrence, with a range of follow-up from 6 to 73 months (average = 32.4 months).

On clinical and pathologic review, the tumors were staged and graded according to criteria of the International Federation of Gynecologists and Obstetricians (Table III). The mean age at presentation for patients with grade 1 tumors was 39.0 years compared with 39.4 years for grade 2 tumors; the mean age at presentation for clinical Stage 1 disease was 38.5 years and for Stage 2 disease it was 42.8 years. Four patients had coincidentally associated gynecologic pathology, as defined in Table IV. The nonmalignant endometrium within the specimen of each case was examined histologically for evidence of precursor lesions to endometrial adenocarcinoma; these results are shown in Table V.

The association between age, smoking history, mean arterial blood pressure, and deviation from ideal body weight was compared with clinical stage and grading of the tumor and precursor endometrial lesions. None of these associations were clinically significant by χ^2 analysis. Nine of 14 cases (63.6%) with an endometrial background of atypical adenomatous hyperplasia occurred in women older than 40 years. This was approaching clinical significance ($p = 0.12$), but there was no established correlation between a precursor lesion and clinical stage or grading. Menstrual bleeding pattern had no correlation with clinical stage or grading, but the presence of atypical adenomatous hyperplasia of the endometrium was strongly associated with heavy, irregular menses ($p = 0.0046$).

Comment

Endometrial adenocarcinoma in young, premenopausal women accounted for 5% of cases in Southern Alberta. This is higher than classically quoted figures but not as high as those of other recent publications.[1-3] This report and others support a trend for increasing incidence with advancing age; the present cohort of patients representing only 5% of reported cases may be attributed to a relatively young and mobile population in Southern Alberta.

An associated diagnosis of the polycystic ovary syndrome may be found in up to 20% to 30% of cases of endometrial adenocarcinoma in young, premenopausal women.[1, 2] In only one of the present 22 cases was there histologic evidence of anovulation. Patient obesity and nulliparity seem to be strongly associated with the development of this cancer in young women[4-8]; this theory is supported in the present review by the finding

that half of the patients were more than 20% heavier than ideal body weight. There are at least two studies, however, to challenge these associations.[3, 8]

The effect of tobacco, if any, is not clear. Some authors[9] claim no association with increased risk of developing endometrial adenocarcinoma, while others maintain smoking will increase the risk in premenopausal women and decrease the risk in postmenopausal women.[10] Exogenous estrogens have been claimed to promote this malignancy, and this may include sequential oral contraceptive pills.[5] The combination oral contraceptive pill has not been implicated directly in this promoter role.[11, 12]

In this study, 21 of 22 patients presented to their primary care physicians with abnormal vaginal bleeding. The most common physical abnormalities were increased uterine size and/or patient obesity. These combinations of signs and symptoms should alert the physician to the possibility of underlying endometrial abnormality, yet the diagnosis of adenocarcinoma was first made after hysterectomy in 22.7% of cases. Although screening the general population with routine endometrial sampling is not cost effective, it should be used more frequently in this selected group of women.[8, 13] As a screening procedure, premalignant pathologic conditions (for instance, endometrial hyperplasia) must be followed with formal cervical dilatation and fractional curettage of the endocervical canal and endometrial cavity.

There was a trend in the study population for atypical adenomatous hyperplasia of the endometrium to occur in women over the age of 40 years, but there was no statistically significant association between "precursor" lesions and adenocarcinoma. Likewise, no statistically significant correlation was found when menstrual pattern, smoking history, age, mean arterial blood pressure, or deviation from ideal body weight was compared with histologic grade or clinical stage of the tumor or precursor endometrial histologic features.

The lack of precursor pathologic conditions (adenomatous hyperplasia) in seven of the 22 patients suggests the possibility that some cases of endometrial adenocarcinoma may arise de novo. The appearance of normally functioning background endometrium, as seen in 15 of the present cases, should not deter the pathologist from diagnosing endometrial adenocarcinoma. As well, it challenges the role of continuous unopposed estrogen in the pathogenesis of this malignancy.

Other investigators[14, 15] have proposed two variants of carcinoma. One variety seems to be estrogen-dependent and is associated with adenomatous hyperplasia, better histologic differentiation-grading, less myometrial invasion, and a better prognosis. The other variety is hormone-independent and carries a worse prognosis. The findings from this present study do not allow identification of such a dichotomous disease process.

Four of the 21 patients available for follow-up in this study did not have surgical castration. Three of these patients had Stage IB, grade 1 tumors and one patient had Stage II, grade 1 disease. These four have been followed up for 6 to 73 months and all remain alive and clinically free of disease to this point. Although bilateral oophorectomy is to be recommended as part of the initial surgical procedure, surgical reexploration, in order to remove the ovaries after hysterectomy alone, is likely unnecessary in patients with early stage disease. A recent article states that endometrial adenocarcinoma does not appear to be a contraindication to estrogen replacement therapy in patients with Stage I disease. Cyclic estrogen plus progestogens may even provide a protective effect.[17] Although specific suggestions regarding the role of ovarian conservation cannot be made from the present study, the routine procedure of bilateral salpingo-oophorectomy is open to question in young women with low-grade, low-stage disease.

Endometrial adenocarcinoma is frequently unsuspected clinically or pathologically in women under the age of 45 years. One may conclude that a greater practice of endometrial sampling is to be encouraged, especially in obese women with abnormal vaginal bleeding and/or an enlarged uterus. The tumor may arise de novo, in some cases in the presence of normal-appearing and functioning endometrium. The pathologist should not be deterred from reporting a diagnosis of endometrial adenocarcinoma in the absence of an identified transition from endometrial hyperplasia to invasive cancer. Although the tumor is usually of low grade and low stage, it may be aggressive and require adjuvant therapy. The implications of long-term estrogen deprivation after hysterectomy and bilateral salpingo-oophorectomy in young patients must be cautiously considered against the unknown risk of ovarian conservation or postoperative estrogen replacement therapy.

REFERENCES

1. Van Nagell JR Jr, Barber RK, eds. Modern concepts of gynecologic oncology. Boston: John Wright-PSG, 1982:6-7, 215-6, 219.
2. Gallup DG, Stock RJ. Adenocarcinoma of the endometrium in women 40 years of age or younger. Obstet Gynecol 1984;64:417.
3. Quinn MA, Kneal BJ, Fortune DW. Endometrial carcinoma in premenopausal women: a clinicopathological study. Gynecol Oncol 1985;20:298.
4. LaVecchia C, Franceschi S, Decarli A, Gallus G, Tognoni G. Risk factors for endometrial cancer at different ages. JNCI 1984;73:667.
5. Henderson BE, Casagrande JT, Pike MC, Mack T, Rosario I, Duke A. The epidemiology of endometrial cancer in young women. Br J Cancer 1983;47:749.

6. Silverberg SG, Makowski EL, Roche WL. Endometrial carcinoma in women under 40 years of age. Cancer 1977; 39:592.
7. Crissman JD, Azoury RS, Barnes AE, Schellhas HF. Endometrial carcinoma in women 40 years of age or younger. Obstet Gynecol 1981;57:699.
8. Koss LG, Schreiber K, Oberlander SG, Moussouris HF, Lesser M. Detection of endometrial carcinoma and hyperplasia in asymptomatic women. Obstet Gynecol 1984; 64:1.
9. Tyler CW Jr, Webster LA, Ory HW, Rubin GL. Endometrial cancer: how does cigarette smoking influence the risk of women under age 55 years having this tumor? Am J Obstet Gynecol 1985;151:899.
10. Smith EM, Sowers MF, Burns TL. Effects of smoking on the development of female reproductive cancers. JNCI 1984;73:371.
11. The Centers for Disease Control, Cancer and Steroid Hormone Study. Oral contraceptive use and the risk of endometrial cancer. JAMA 1983;249:1600.
12. Trapido EJ. A prospective cohort study of oral contraceptives and cancer of the endometrium. Int J Epidemiol 1983;12:297.
13. Grimes DA. Diagnostic dilatation and curettage: a reappraisal. Am J Obstet Gynecol 1982;142:1.
14. Deligdisch L, Cohen CJ. Histologic correlates and virulence implications of endometrial carcinoma associated with adenomatous hyperplasia. Cancer 1985;56:1452.
15. Bokhman JV. Two pathogenetic types of endometrial cancer. Gynecol Oncol 1983;15:10.
16. Peters WA III, Andersen WA, Thornton WN Jr, Morley GW. The selective use of vaginal hysterectomy in the management of adenocarcinoma of the endometrium. Am J Obstet Gynecol 1983;146:285.
17. Creasman WT, Henderson D, Hinshaw W, Clarke-Pearson DL. Estrogen replacement therapy in the patient treated for endometrial cancer. Obstet Gynecol 1986; 67:326.

Uterine sarcoma: An analysis of 74 cases

Allan L. Covens, M.D., Jeffrey A. Nisker, M.D., William B. Chapman, M.D., and H. Hugh Allen, M.D.

London, Ontario, Canada

In order to determine whether recent methods of diagnosis and treatment have resulted in an improved survival for patients with uterine sarcoma, we reviewed 99 cases treated in our center from 1970-1985. Morphologic characteristics of 74 tumors were specifically reassessed for this study. All tumors were graded. Of 42 Stage I cases that were morphologically assessed, tumor-positive pelvic lymph nodes were found in two of the 15 patients in whom sampling was done. No cases of tumor-positive para-aortic nodes were found in 14 patients with Stage I disease. In Stage I and Stage II, no cases of positive para-aortic nodes were found in association with negative pelvic nodes. The 2- and 5-year survival rates in Stage I were 47.4% and 29.4%, respectively. Local recurrence decreased ($p < 0.01$) in Stage I from nine of 22 cases in which operation alone was performed to none of 15 cases in which pelvic radiotherapy was added, but no improvement in the 5-year survival rate was observed. As with lymphadenectomy and radiotherapy, the recent use of chemotherapy for uterine sarcoma had no impact on survival. (Am J Obstet Gynecol 1987;156:370-4.)

Key words: Uterine sarcoma, diagnosis, treatment, survival

Sarcomas of the uterus account for 3% to 5% of uterine malignancies.[1-4] This infrequent occurrence, as well as the multiple morphologic features, has made study and recommendations for treatment difficult. In recent years lymphadenectomy, adjuvant radiotherapy, and chemotherapy have been suggested as possible therapeutic modalities that might improve the generally poor prognosis. We thus evaluated these methods for the treatment of uterine sarcoma in a large regional cancer center.

From the Departments of Obstetrics and Gynecology and Pathology, University of Western Ontario and Ontario Cancer Treatment and Research Foundation, London Clinic.
Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.
Reprint requests: H. H. Allen, M.D., Victoria Hospital, Department of Obstetrics and Gynecology, 800 Commissioners Road, East, Box 5375, London, Ontario, Canada N6A 4G5.

Material and methods

Ninety-nine patients were diagnosed as having uterine sarcoma at the Ontario Cancer Treatment and Research Foundation London Clinic from 1970 to 1985. Data regarding presenting complaint, previous pelvic radiation, diagnostic procedure, clinical and surgical stage, histologic diagnosis, surgical treatment, and adjuvant radiotherapy or chemotherapy as well as recur-

Table I. Morphologic features and percent survival

	N	Stage				Overall survival (%)	
		I	II	III	IV	2 yr	5 yr
Malignant mixed müllerian tumor							
Homologous	28	10	6	6	6	24.0	4.3
Heterologous	15	13	1	0	1	33.3	33.3
Total	43	23	7	6	7	27.0	14.3
Endometrial stromal sarcoma							
Low-grade	3	3	0	0	0	100.0	100.0
High-grade	4	2	0	1	1	25.0	25.0
Total	7	5	0	1	1	57.1	57.1
Leiomyosarcoma							
Atypia							
Mild	5	3	0	1	1	80.0	40.0
Moderate	9	7	0	1	1	44.4	12.5
Severe	3	1	1	0	1	50.0	0
Mitotic count							
5-9/10 hpf	4	3	0	0	1	75.0	50.0
10-20/10 hpf	10	5	1	2	2	66.7	14.2
>20/10 hpf	3	3	0	0	0	0	0
Total	17	11	1	2	3	57.1	16.7
Adenosarcoma	1	0	1	0	0	0	0
Mixed sarcoma	1	1	0	0	0	0	0
Unclassified	5	2	0	1	2	0	0

hpf = High-power field.

rence were retrieved. Information not present in the initial chart review was added from referring physician records, other cancer treatment center's records, individual hospital charts, and personal telephone calls to the patients. Follow-up was 100%.

Clinical stage was based on guidelines of the International Federation of Gynecologists and Obstetricians for uterine neoplasms. Pathologic material was reviewed specifically for this study by one of the authors (W. B. C.) using the classification of Hendrickson and Kempson.[5] Low-grade endometrial stromal sarcomas (endolymphatic stromal myosis) were included. The criteria for diagnosis of leiomyosarcoma were >10 mitotic figures per 10 high-power fields or five to 10 mitotic figures per 10 high-power fields with moderate or severe atypia. An average of 11 blocks of primary tumor had been prepared per case, and the range was seven to 21. At least one slide stained with hematoxylin and eosin was reviewed for each block, and in some cases special stains were also available for review. A mitotic count was done for all cases; this was reported as the number of mitotic figures per 10 high-power fields, with the use of a 40× objective and 10× ocular. At least 10 high-power fields in the most cellular part of the tumor were examined in each case. Atypia was graded as mild, moderate, or severe.

Groups were compared with the use of χ^2 analysis and means were compared with Student's t test. The 2- and 5-year survival rates were reported as crude survivals.

Table II. Clinical and surgical stage

Stage	Clinical		Surgical	
	n	%	n	%
I	56	75.7	42	52.2
II	5	1.4	9	12.7
III	5	6.8	7	9.9
IV	8	11.3	13	18.3
	74		71*	

*Three patients underwent curettage only.

Results

Of the 99 cases initially diagnosed as uterine sarcoma, 25 were excluded before pathologic review because of unavailability of slides. All of the original diagnoses for the remaining 74 cases were confirmed. As shown in Table I, malignant mixed müllerian tumors accounted for 58.1% of all tumors. The tumor grading is also presented in Table I.

The mean age of the patients was 61.9 years, and the ages ranged from 15 to 92 years. The mean ± SD ages by histologic types were: malignant mixed müllerian tumor, 65.8 ± 13.0 years; endometrial stromal sarcoma, 55.1 ± 17.4 years; leiomyosarcoma, 55.2 ± 11.5

Table III. Lymph node status

	Stage I				*Stage II*			
	Pelvic		*Para-aortic*		*Pelvic*		*Para-aortic*	
	Positive	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*
n	2	13	0	14	4	0	2	0
Survival (%)								
2 yr	0	50.0	—	41.7	0	—	0	—
5 yr	0	25.0	—	22.2	0	—	0	—

Table IV. Survival versus stage

Stage	*n*	*Survival (%)*	
		2 yr	*5 yr*
I	42	47.4	29.4
Malignant mixed müllerian tumor	23	31.6	23.5
Endometrial stromal sarcoma	5	80.0	75.0
Leiomyosarcoma	11	72.7	33.3
Others	3	0	0
II	9	25.0	12.5
III	10	20.0	0
IV	13	9.1	0

years; others, 60.6 ± 13.1 years. The mean age of patients with leiomyosarcoma was significantly younger than that of patients with malignant mixed müllerian tumors ($p < 0.01$). The most common presenting complaints were abnormal vaginal bleeding (79.7%), pelvic or abdominal pain (16.2%), an enlarged uterus (12.2%), vaginal discharge (9.5%), and abdominal or pelvic mass (9.5%).

The clinical and surgical staging is described in Table II. At the time of operation, 23.2% of patients with clinical Stage I disease were noted to have more advanced disease.

Pelvic lymph node metastases were found in two of 15 Stage I cases, but no para-aortic nodal metastases were found in 14 patients (Table III). There were no cases of positive para-aortic nodes with negative pelvic nodes (n = 13). In Stage II, tumor-positive nodes were found in all four cases where pelvic nodes were removed and in both cases where para-aortic lymphadenectomy was performed. In Stages I and II malignant mixed müllerian tumors accounted for 83.3% of cases with tumor-positive lymph nodes. In both Stage I and Stage II there were no 2-year survivors in any of the patients with tumor-positive pelvic or para-aortic lymph nodes. In Stage I, the 5-year survival rate for patients with negative nodes (25.0%) was not significantly different from that in those cases where the lymph nodes were not sampled (34.8%).

The 2- and 5-year survival rates by stage are shown in Table IV. The overall crude 2- and 5-year survivals were 33.8% and 17.5%, respectively, and 47.4% and 29.4%, respectively, for Stage I. Fifty-one patients have died, with all deaths occurring within 5 years of initial operation.

Survival rates in Stage I for the various treatment regimens are described in Table V. Although there was a significant decrease ($p < 0.01$) in local recurrence with the addition of radiation to operation (none of 15) compared with that in operation alone (nine of 22), the 2- and 5-year survivals were similar. When simple hysterectomy is compared with radical hysterectomy in patients with Stage I disease (having no additional therapy), the 2- and 5-year survivals were not significantly different (Table V).

Twenty-four patients received chemotherapy. The various regimens included: vincristine, actinomycin D, and cyclophosphamide (eight patients); cyclophosphamide, vincristine, Adriamycin, and dimethyl-triazeno imidazole carboxamide (seven patients); and cisplatin-based therapies (five patients). Adriamycin, in addition to other chemotherapeutic agents, was given to 15 patients. No patient received single-agent chemotherapy. No change in survival was observed with the addition of chemotherapy.

There have been tumor recurrences in 35 patients and second recurrences in 11. Recurrences were observed within 9 months in 54.3% of cases, and all recurrences observed were within 4 years of initial diagnosis. As noted in Table VI, 51.4% of first recurrences involved the pelvis, with 45.7% involving the pelvis only. The second most common site of initial recurrence was the lung (31.4%), with 22.9% being pulmonary only. The abdomen was involved in 20.0% in recurrences.

Survival according to histologic type is given in Table I. When the various morphologic types are compared (Table I), no significant differences in survival were noted, with the exclusion of low-grade endometrial stromal sarcoma. All malignant mixed müllerian tumors showed areas of severe atypia and high mitotic counts in both the sarcomatous and carcinomatous areas. The same was true for the sarcomatous portions

Table V. Stage I: Primary therapy and survival

	Simple hysterectomy	*Radical hysterectomy*	*Operation plus radiation*	*Radiation plus chemotherapy*	*Operation plus radiation plus chemotherapy*
n	17	5	15	4	1
Survival (%)					
2 yr	43.8	50.0	46.2	75.0	0
5 yr	25.0	33.3	41.7	50.0	0

of the unclassified sarcomas, the mixed sarcoma, and the adenosarcoma. Leiomyosarcoma was analyzed both by degee of atypia and by mitotic count. Thirteen cases of leiomyosarcoma had >10 mitoses per 10 high-power fields and four cases had five to nine mitoses per 10 high-power fields with moderate to severe atypia. There was a better 5-year survival with lower mitotic counts and less atypia, but the small numbers preclude significance testing.

Four patients had previous pelvic radiation, with the average time from radiation to diagnosis being 16 years, (range 5 to 27 years). The indications for the previous radiation included: cervical carcinoma (n = 2), bladder carcinoma (n = 1), and ovarian carcinoma (n = 1). Morphologically, three were malignant mixed müllerian tumors and the other was an adenosarcoma.

Table VI. Site of first recurrence

Site	*n*	*%*
Pelvis		
Isolated	16	45.7
Total	18	51.4
Lung		
Isolated	8	22.9
Total	11	31.4
Abdomen	7	20.0
Other sites	4	11.4

Comment

The 2- and 5-year survivals for Stage I reported in this paper are comparable with those of previous studies[3] but less than those of others.[1, 6-8] The single most important predictor of survival is stage. In this study, grade appears to be of use prognostically for endometrial stromal sarcoma and possibly leiomyosarcoma. The usefulness of grading in endometrial stromal sarcoma is widely accepted[9-11] and a correlation between grade and prognosis has been shown for leiomyosarcoma.[12] Conversely, grading of malignant mixed müllerian tumor does not offer any prognostic value,[12, 13] a conclusion with which we agree.

In this study, as in other studies addressing lymphadenectomy for uterine sarcoma,[6, 14] small numbers preclude meaningful conclusions. The fact that five of six patients with positive nodes had morphologic features of malignant mixed müllerian tumor agrees with a previous study[6] stating that malignant mixed müllerian tumors have the most propensity to spread via the lymphatics. As no patient with tumor-positive lymph nodes survived 2 years, lymphadenectomy appears to be of limited usefulness.

The incidence of sarcoma in patients with a history of previous pelvic radiation is comparable to that of previous reports.[1, 2, 15] Rarely is radiation used for benign disease today, but the numerous indications in the past may be one cause of the higher incidence reported in previous studies.[6, 16] The higher proportion of malignant mixed müllerian tumors observed in this study is consistent with that of previous reports.[2, 15]

The inaccuracies of clinical staging are evident in the fact that 23.2% of Stage I cases were understaged when intraoperative findings were evaluated. The high proportion of distant recurrences (28.6%) in Stage I disease further emphasizes the need for systemic therapy as part of the primary treatment plan. The propensity for pulmonary recurrences (31.4% overall and 22.9% as an isolated location) further emphasizes the need for adjuvant chemotherapy. The aggressiveness of uterine sarcomas is further reflected by the fact that more than 50% of recurrences developed within 9 months of initial treatment. Low-grade endometrial stromal sarcoma, however, behaves differently from other uterine sarcomas in its tendency to recur after many years. Comparisons of primary treatment modalities could be addressed only in Stage I and II disease in this study. As in previous reports,[1-3, 7, 8] the addition of radiotherapy did not significantly improve survival. Despite the adjuvant use of chemotherapy in 14.9% of our patients, neither survival rate nor recurrence interval was improved.

The correlation of negative pelvic lymph nodes with negative para-aortic nodes questions the value of paraaortic lymphadenectomy. Similarly the fact that there were no survivors with tumor-positive pelvic lymph nodes demonstrates the futility of therapeutic lymphadenectomy in uterine sarcoma. Radical hysterectomy has not been found to improve survival in Stage I disease and therefore has no role in uterine sarcoma ex-

cept possibly for endometrial stromal sarcoma with potential parametrial involvement.

The addition in recent years of radiotherapy, lymphadenectomy, and chemotherapy to hysterectomy for uterine sarcoma has not impacted on the poor prognosis. More effective chemotherapeutic agents would seem the most appropriate direction for future studies of uterine sarcoma.

REFERENCES

1. Salazar OM, Bonfiglio TA, Patten SF, et al. Uterine sarcomas. Natural history, treatment and prognosis. Cancer 1978;42:1152.
2. Wheelock JB, Krebs HB, Schneider V, Goplerud DR. Uterine sarcoma: analysis of prognostic variables in 71 cases. AM J OBSTET GYNECOL 1985;151:1016.
3. Shimm DS, Bell DA, Fuller AF, et al. Sarcomas of the uterine corpus: prognostic factors and treatment. Radiother Oncol 1984;2:201.
4. Cavanagh D, Praphat H, Ruffolo EH. Sarcomas of the uterus. Obstet Gynecol Ann 1979;8:413.
5. Hendrickson MR, Kempson RC. Surgical pathology of the uterine corpus. In: Bennington SL, ed. Major problems in pathology. Philadelphia: WB Saunders, 1980, vol 12: 419.
6. Peters WA, Kumar NB, Fleming WP, Morley GW. Prognostic features of sarcomas and mixed tumors of the endometrium. Obstet Gynecol 1984;63:550.
7. Sorbe B. Radiotherapy and/or chemotherapy as adjuvant treatment of uterine sarcomas. Gynecol Oncol 1985; 20:281.
8. Hannigan EV, Freedman RS, Rutledge FN. Adjuvant chemotherapy in early uterine sarcoma. Gynecol Oncol 1983;15:56.
9. Norris HJ, Taylor HB. Mesenchymal tumors of the uterus. I. A clinical and pathological study of fifty-three endometrial stromal tumors. Cancer 1966;19:755.
10. Jensen PA, Dockerty MB, Symmonds RE, Wilson RB. Endometrioid sarcoma ("stromal endometriosis"). Report of fifteen cases including 5 with metastases. AM J OBSTET GYNECOL 1966;95:79.
11. Yoonessi M, Hart WR. Endometrial stromal sarcomas. Cancer 1977;40:898.
12. Saksela E, Lampinen V, Procope BJ. Malignant mesenchymal tumors of the uterine corpus. AM J OBSTET GYNECOL 1974;120:452.
13. Chuang JT, Vanvelden DJJ, Graham JB. Carcinosarcoma and mixed mesodermal tumour of the uterine corpus. Review of 49 cases. Obstet Gynecol 1970;35:769.
14. Disaia PJ, Morrow CP, Boronow R, Creasman W, Mittelstaedt L. Endometrial sarcoma: lymphatic spread pattern. AM J OBSTET GYNECOL 1973;130:104.
15. Norris HJ, Taylor HB. Post radiation sarcomas of the uterus. Obstet Gynecol 1965;26:689.
16. Aaro LA, Symmonds RE, Dockerty MB. Sarcoma of the uterus. A clinical and pathological study of 177 cases. AM J OBSTET GYNECOL 1966;94:101.

Laser surgery for vulvar intraepithelial neoplasia: Principles and results

V. C. Wright, M.D., and Eleanor Davies, M.D.
London, Ontario, Canada

Twenty-nine patients with grade 3 vulvar intraepithelial neoplasia were treated with the carbon dioxide laser while they were under anesthesia. Superficial lesions in nonhairy areas were vaporized to <1 mm, but lesions extending onto hairy areas were vaporized to 3 mm to destroy disease in skin appendages. Eighty-three percent of lesions involved nonhairy areas alone. Seventy-two percent of cases were cured by one laser application, 25% by two, and one case by three. Two patients had permanent hair loss after healing. The laser is an appropriate modality for lesions in nonhairy areas but scalpel excision is probably better for vulvar intraepithelial neoplasia in hairy areas. (AM J OBSTET GYNECOL 1987;156:374-8.)

Key words: Laser surgery, vulvar intraepithelial neoplasia, vulvar carcinoma in situ

Vulvar intraepithelial neoplasia has been considered a rare disease of early menopausal and postmenopausal women. Today, however, it is being seen more commonly in young, reproductive-aged women. Many of the women having a diagnosis of vulvar intraepithelial neoplasia have a history of vulvar viral infections, particularly human papillomavirus.[1] This fact and other evidence has led investigators to suspect a cause-and-effect relationship.[2-5] Vulvar intraepithelial neoplasia can be found anywhere on the vulva. Most lesions occupy nonhairy areas, but they can also occur in hairy areas.[6] As many as 50% of cases are multifocal areas,[6] and lesions can extend from one area onto another,

From the Department of Obstetrics and Gynecology and the Department of Pathology, The University of Western Ontario.
Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.
Reprint requests: V. C. Wright, M.D., 887 Richmond St., London, Ontario, Canada N6A 3J1.

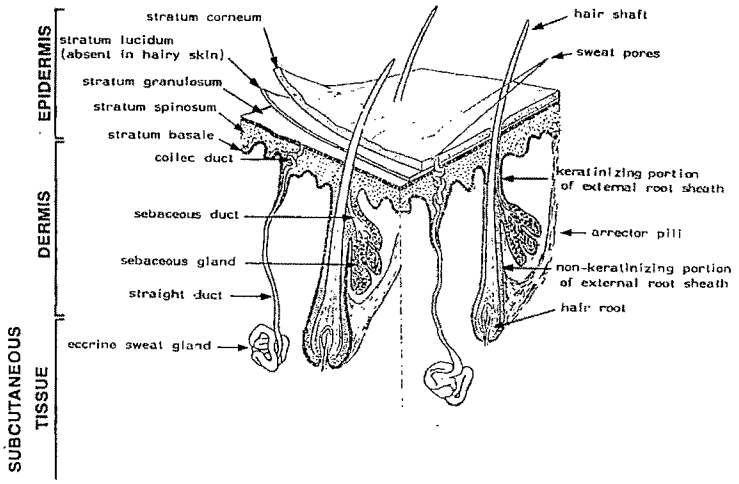


Fig. 1. Histologic structural relationship of the sebaceous gland, hair follicle, and exocrine sweat gland.

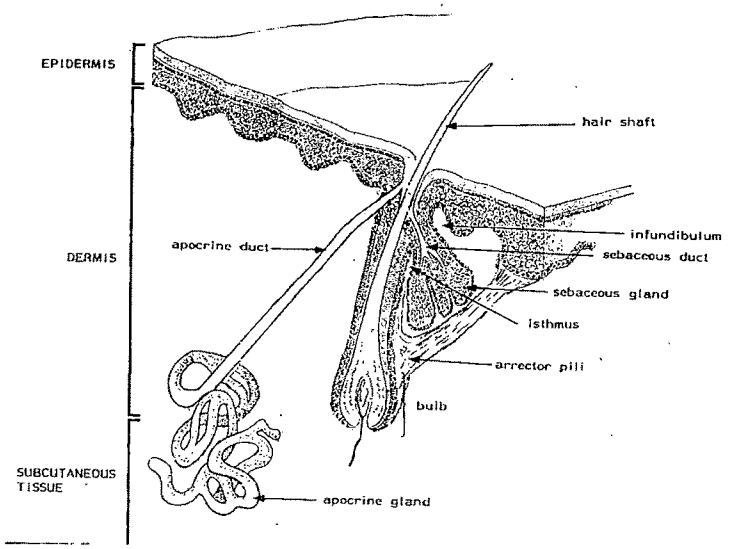


Fig. 2. Histologic structural relationship of the sebaceous gland, hair follicle, and sweat gland.

such as from the vulva to the perianal skin (17% of cases) or clitoral prepuce (18% of cases).[1]

Treatments by simple excision, simple vulvectomy, skinning vulvectomy, cryosurgery, and topical 5-fluorouracil have been disappointing.[1, 6-8] Recurrence is the same, about 30%, after local incision and simple vulvectomy, and in 39% of cases recurrence is found at the margin after skinning vulvectomy.[9] As many as 48% of cases of multifocal disease recur.[10] Topical 5-fluorouracil is frequently too irritating for patients to continue the 4- to 6-week regimen.[6, 9] Cryosurgery simply does not work[1] and repeated surgical procedures often produce unsatisfactory cosmetic results.

The apparently rising incidence of vulvar intraepithelial neoplasia in younger, sexually active women and the need for re-treatment in many patients have motivated some surgeons to use the carbon dioxide laser as a surgical modality for this disease. This has met with varying success, ranging from acceptable[11] (73% disease free after one to three laser applications) to dismal[12] (74% of one series of patients had three to six laser applications and almost 10% of the women still had disease), and complications of bleeding and infection have been reported.[11, 13, 14] The purpose of this paper is to illustrate that satisfactory cure rates, few complications, and excellent cosmetic results can be obtained for both unifocal and multifocal grade 3 vulvar intraepithelial neoplasia disease with the carbon dioxide laser. The operative approaches are based on the histologic depths of disease in different areas of the vulva and the potential for vulvar intraepithelial neoplasia to involve underlying adnexal structures.

Table I. Postoperative care after vulvar laser surgery

1.	Tea sitz baths or warm tea bag compresses four times a day
2.	Tannic acid ointment (composed of 5% tannic acid and 95% PCCA concentrate* or 5% tannic acid and 95% petrolatum supplied in 50 to 100 gm container) applied to the wound three times a day
3.	One quarter strength hydrogen peroxide sitz baths as needed (one part hydrogen peroxide and four parts water), should a cleansing agent be necessary
4.	Use of a hair dryer to dry the skin after bathing and sitz baths
5.	Silver sulfadiazine applied topically as necessary to control superficial bacterial infection
6.	Analgesics as necessary
7.	Avoidance of constricting clothing
8.	No intercourse until the area is healed

*Professional Compounding (Canada), Inc., 3 Centre St., London, Ontario, Canada N6J 1T4.

Structure of the skin

To eliminate vulvar intraepithelial neoplasia it is necessary to understand the structure of the skin and its underlying appendages and recognize which ones are likely to be become involved by disease and to what depth (Figs. 1 and 2). The structures in question are the external hair root sheath, the sebaceous gland and its duct, and the apocrine and eccrine glands and ducts. The external hair root sheath is a downward continuation of the epidermis. To the isthmus, including the entrance of the sebaceous duct, the squamous epithelium is stratified and produces keratin in the same manner as the vulvar skin surface. Below this level the sheath is not stratified and does not produce keratin. Because the upper parts of the hair root sheath and the lining of the sebaceous gland duct are contiguous with the surface epithelium and are composed of similar cells, these areas are susceptible to extension of disease.[15] Involvement of hair root sheaths to depths of 0.8 to 2.5 mm has been documented.[16] Involvement of the sebaceous duct, which is secondary to that of the sheath, occurs, although less often. The ducts of the eccrine and apocrine glands are lined by their own independent epithelium, which is not contiguous with the surface epithelium. Because of this these structures are far less likely to be involved. In the rare instance when disease has appeared to involve these ducts it has been to depths of ≤0.5 mm (Ferenczy A., personal communication, 1985).

Rationale for laser surgery

The reasons for using the carbon dioxide laser are many. This procedure requires microscopic scrutiny of disease because it is performed through an operating microscope. Normal tissue is left undisturbed and inflammatory response is reduced. The procedure usually does not produce bleeding and can be done on an outpatient basis with small lesions. Re-treatment can be performed, if needed, and exceptional cosmetic and functional results can be achieved. However, the latter advantage does not apply to all locations of vulvar intraepithelial neoplasia.

Because different areas of the vulva have different adnexal structures (hair follicles, sebaceous glands, apocrine and exocrine glands), the depth of disease and the technique of removing it with the carbon dioxide laser must vary. In some areas the carbon dioxide laser may not be the most appropriate surgical modality. For best management, the vulva, perineum, and anus should be divided into hairy and nonhairy areas.

Nonhairy areas. The nonhairy sites include the clitoris, labia minora, and inner surface of the labia majora, posterior fourchette, and anus. Since hair shafts are absent in these areas, it is unlikely that vulvar intraepithelial neoplasia will extend beyond the thickness of the epidermis (≤500 to 600 μm). Therefore, laser vaporization should be superficial and include the thickness of the epidermis only. This results in a histologic depth of <1 mm. Some heat conduction will extend the injury into the underlying superficial dermis. This may result in characteristic edema, blister formation, and pain. Such an injury may require 21 to 28 days to heal, but scarring should not occur with epithelial vaporization only.

Hairy areas. These areas are the outer surface of the labia majora and more lateral perineal and perianal areas. In these areas vulvar intraepithelial neoplasia can extend down the hair shaft (external hair sheath) and its associated sebaceous gland duct into the dermis as much as 2.5 mm. To eradicate disease in hairy areas, therefore, vaporization into the dermis of 3 mm is required to account for potential extension of the vulvar intraepithelial neoplasia along the hair shaft and its associated adnexal structure (duct of sebaceous gland). The amount of dermis lost depends on the site and extent of vulvar intraepithelial neoplasia involvement. This full-thickness vaporization results in destruction of the epidermis, dermis, and some complete epidermal structures.

Methods and material

During 1981 to 1985, 29 patients were referred to the Woman's Clinic at St. Joseph's Hospital in London, Ontario, Canada, for management of grade 3 vulvar intraepithelial neoplasia (severe dysplasia and carcinoma in situ). The majority of these patients had previous biopsies, either by the Keyes punch, by punch biopsy, or by incomplete local scalpel excision. A thorough colposcopic examination of the cervix, vagina, vulva, perineum, and anal area was carried out at the clinic. The appropriate areas were stained with acetic

Table II. Distribution of disease

Lesion location	Nonhairy		Hairy		Hairy and nonhairy		Total	
	No.	%	No.	%	No.	%	No.	%
Upper one-third	4	13.8	0	0	0	0	4	13.8
Posterior one-third	9	31.0	0	0	1	3.5	10	34.5
Posterior two-thirds	9	31.0	0	0	0	0	9	31.0
Multifocal	2	6.9	0	0	4	13.8	6	20.7
Total	24	82.8	0	0	5	17.2	29	100

Table III. Treatment success

Lesion location	Cured by one procedure		Cured by two procedures		Cured by three procedures		Overall cures	
	No.	%	No.	%	No.	%	No.	%
Nonhairy areas								
Upper one-third	4	100	—	—	—	—	4	100
Posterior one-third	9	100	—	—	—	—	9	100
Posterior two-thirds	5	55.6	4	44.4	—	—	9	100
Multifocal	0	0.0	2	100	—	—	2	100
Multifocal, hairy and nonhairy	2	50.0	1	25.5	1	25.5	4	100
Posterior one-third, hairy and nonhairy	1	100	—	—	—	—	1	100
Total	21	72.4	7	24.1	1	3.5	29	100

acid. Toluidine was not used because we believe that acetic acid with magnification is more reliable. Frequently further biopsies were necessary to be sure that no invasive disease was present. A complete mapping of the disease was recorded.

All patients were under general anesthesia while they were treated. A surgical laser coupled to an operating microscope was used to produce an effective beam diameter of 2 mm with power density ranging from 650 to 1200 W/cm^2. Lesions were first outlined incorporating a colposcopically normal peripheral margin of 2 to 3 mm. Vaporization was carried out to a depth of just less than 1 mm in nonhairy areas and to 3 mm in hairy areas to ensure all disease was eradicated. Carbonized debris was then removed by irrigation or with a wet sponge. The vaporized defects were left to granulate and epithelialize.

Patients were given specific instructions for postoperative care. These are outlined in Table I. They were cautioned that infection and temperature elevation were possible and that they should call the office if either occurred. They were instructed to gently separate opposing lasered labia at least once each day during a sitz bath to prevent coaptation. They were informed that satisfactory healing might take 4 to 6 weeks after superficial vaporization and as long as 2 months after vaporization into the dermis. Patients were advised to avoid intercourse until satisfactory healing had taken place. Finally, they were warned that loss of pigmentation and alopecia might occur in areas of deep vaporization.

The initial postoperative evaluation was scheduled for 4 weeks after operation, the next at 3 months, and the third at 6 months. If a 6-month assessment revealed normal colposcopic findings, the patient was instructed to contact her referring physician for yearly examinations. A progress note was then sent to the clinic by the examining physician.

Results

In 83% of cases the grade 3 vulvar intraepithelial neoplasia lesions involved nonhairy areas only. In 17% lesions involved hairy and nonhairy areas. No lesions involved hairy areas alone (Table II). The most common single site was the posterior fourchette (52% of cases). The clitoris was involved in 6.9%. The majority of lesions occupied the posterior two thirds of the vulva. One patient had perianal extension of the disease.

Fourteen of 29 patients (48.3%) had a history of condyloma of the vulva. Four (13.8%) had condyloma associated with vulvar intraepithelial neoplasia at the time of treatment. Seven patients (24.1%) had a history of cervical intraepithelial neoplasia: grade 2 in three cases and grade 3 in four. No patient had a history of vaginal intraepithelial neoplasia. Twenty-seven of the 29 patients (93.1%) smoked.

Tea sitz baths and topical 5% tannic acid combined with 95% petrolatum were very effective in controlling pain. Laser surgery produced considerable pain and edema on the labia minora and three patients required a Foley catheter for as long as 10 days.

Two patients with vulvar intraepithelial neoplasia extending onto hairy areas healed with loss of hair. All other patients had satisfactory cosmetic results. No patient developed a systemic infection.

The success of laser surgery by site is shown in Table III. Most cases (72.4%) were cured by one laser procedure. One quarter of the cases required two laser procedures and one case required three treatments. In all cases the original lesions were eradicated. Seventy-five percent of lesions in nonhairy areas alone were cured by one laser application whereas only 60% of lesions that extended onto hairy areas were cured by one laser procedure.

At this writing two of the 29 patients (6.9%) who had normal findings 1 year after operation developed new disease. One had two laser procedures for a lesion in the posterior two thirds and she developed a new lesion in the upper third of one side of the vulva. This lesion was excised with a scalpel by another gynecologist two years after the laser procedure. One year later this same patient developed another lesion on the opposite side of the vulva in the upper third. She is presently contemplating having this area removed by laser. Another patient with a lesion in the posterior two thirds developed a new lesion adjacent to the site of laser treatment 3 years later. This was successfully removed by laser vaporization.

Comment

Baggish and Dorsey,[12] in an early investigation of laser surgery for vulvar intraepithelial neoplasia, recommended a uniform depth of vaporization of 3 mm for all areas of the vulva. However, because the labia minora and clitoris can be thinner than 3 mm, they might be destroyed by such depths of destruction. Furthermore, vaporization to that depth is unnecessary as well as hazardous in some areas, since adnexal structures that might be involved are absent in the nonhairy areas of the vulva where most lesions arise. In these nonhairy areas superficial ablation of the epithelium will be sufficient to eliminate the disease quickly, easily, and effectively, usually with one treatment. This has been noted by others.[14] The beam's slight thermal effect on adjacent and underlying normal tissue is enough to seal small blood vessels and lymphatics without interfering with healing. This enables the procedure to be performed without bleeding, prevents infection, and allows the vulva to heal without scarring.

Hairy areas, however, can be involved into the papillary and possibly the reticular dermis. Elimination of disease requires deep vaporization, which can cause delayed healing, scarring, and alopecia. We therefore advocate excising lesions in the hairy areas by scalpel. This approach to operation for grade 3 vulvar intraepithelial neoplasia, on the basis of the histologic depth of disease, is a reasonable alternative to more radical surgical procedures and seems a logical way of eliminating lesions while minimizing complications. This concept of histologic depth is consistent with the vaporization planes recently described by Reid[17] and warrants further investigation.

REFERENCES

1. Friedrich EG. Carcinoma in situ of the vulva: a continuing challenge. Am J Obstet Gynecol 1980;136:830.
2. Woodruff JD, Julian C, Puray T, Mermut S, Katayama P. The contemporary challenge of carcinoma in situ of the vulva. Am J Obstet Gynecol 1973;115:677.
3. Lupulescu A, Mehregan AH, Rahbari H, Pinkus H, Birmingham DJ. Venereal warts vs Bowen disease. JAMA 1977;237:2520.
4. Kovi J, Tillman RL, Lee SM. Malignant transformation of condyloma acuminatum. Am J Clin Pathol 1974; 61:702.
5. Jagella HP, Stegner HE. Histopathologic and cytophotometric study of dysplastic and precancerous condylomata acuminata. Arch Gynaekol 1974;216:119.
6. Forney JP, Morrow CP, Townsend DE, DiSaia PJ. Management of carcinoma in situ of the vulva. Am J Obstet Gynecol 1977;127:801.
7. Kaufman RH, Gardner HL. Intraepithelial neoplasia of the vulva. Clin Obstet Gynecol 1965;8:1035.
8. Buscema BA, Woodruff DJ, Parmley TH, Genadry R. Carcinoma in situ of the vulva. Obstet Gynecol 1980; 55:225.
9. DiSaia PJ, Rich WM. Surgical approach to multifocal carcinoma in situ of the vulva. Am J Obstet Gynecol 1981;140:136.
10. Japaze H, Garcia-Bunuel R, Woodruff JD. Primary vulvar neoplasia: a review of in situ and invasive carcinoma, 1935-1972. Obstet Gynecol 1977;49:404.
11. Ferenczy A. Using the laser to treat vulvar condylomata acuminata and intraepidermal neoplasia. Can Med Assoc J 1983;128:135.
12. Baggish MS, Dorsey JH. CO_2 laser for the treatment of vulvar carcinoma in situ. Obstet Gynecol 1981;57:371.
13. Baggish MS. Complications associated with carbon dioxide laser surgery in gynecology. Am J Obstet Gynecol 1981;139:568.
14. Townsend DE, Levine RU, Richart RM, Crum CP, Petrilli ES. Management of vulvar intraepithelial neoplasia by carbon dioxide laser. Obstet Gynecol 1982;60:49.
15. Moschella SL, Hurley HJ. Dermatology. 2d ed. Toronto, Ontario, Canada: WB Saunders, 1985, vol 1:59-63.
16. Mene A, Buckley CH. Involvement of the vulval skin appendages by intraepithelial neoplasia. Br J Obstet Gynaecol 1985;92:634.
17. Reid R. Superficial laser vulvectomy. III. A new surgical technique for appendage-conserving ablation of refractory condylomas and vulvar intraepithelial neoplasia. Am J Obstet Gynecol 1935;152:504.

POSTGRADUATE OBSTETRICS & GYNECOLOGY

A BIWEEKLY REVIEW OF CLINICAL OBSTETRICS AND GYNECOLOGY

Editors

Editor-in-Chief
EDWARD E. WALLACH, M.D.

Associate Editor
ROGER D. KEMPERS, M.D.

Associate Editor
RONALD J. BOLOGNESE, M.D.

Editorial Board

HERBERT J. BUCHSBAUM, M.D.
CHARLES B. HAMMOND, M.D.
NATHAN KASE, M.D.
JOHN J. MIKUTA, M.D.
JENNIFER R. NIEBYL, M.D.
JOHN T. QUEENAN, M.D.
RICHARD H. SCHWARZ, M.D.
LEON SPEROFF, M.D.

A CONTINUING AMA CATEGORY I MEDICAL EDUCATION PROGRAM

Postgraduate Obstetrics and Gynecology is a biweekly publication consisting of 26 lessons per year. Every two weeks during the calendar year each subscriber receives a state-of-the-art program which contains an original article focusing on a basic facet or contemporary problem and a self-test. Each article is prepared by an authority from among our distinguished editorial board or by a guest author selected by our editors. The articles are timely, authoritative, concise, and supplemented by visuals to provide clear illustrations of important details.

Each program concludes with a series of multiple choice questions which crystallize the most significant points of the article. The correct responses for each lesson are contained in the subsequent issue.

Accreditation—As an organization for continuing medical education, the University of Pennsylvania School of Medicine designates this continuing medical education activity as meeting the criteria for 1 credit hour per biweekly issue in Category 1 for Education Materials for the Physician's Recognition Award of the American Medical Association provided it has been completed according to instructions. This course has also been approved by the Americal College of Obstetricians and Gynecologists for 1 cognate per biweekly issue formal learning, as well as by the Committee on Continuing Education of the American Osteopathic Association for 1 credit hour per biweekly issue in Category 2B.

Each paid subscription is entitled to 26 CME credits and written CME certification is available upon request.

Please enter my subscription for one year (26 issues) of **Postgraduate Obstetrics & Gynecology.** I understand that my subscription will start with the issue following receipt of my payment ($160.00).

½ year subscription available—$90 for 13 issues.

(*Overseas orders* please add $35.00 for Air Mail orders.)

(check one)
☐ check enclosed
☐ bill me

Mail to:
POBG
P.O. Box 23263
Baltimore, MD 21203-9990

Name _____

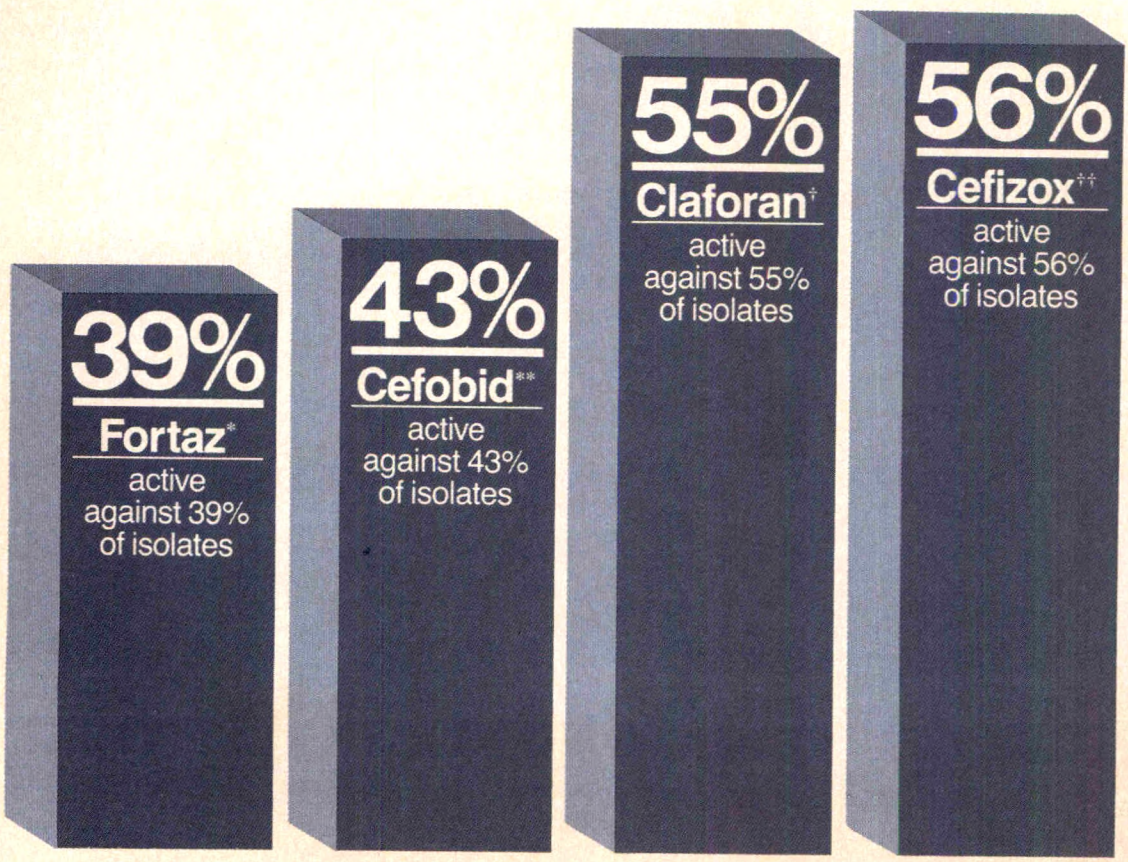
Address _____

City _____ State _____ Zip _____

Postgraduate Obstetrics and Gynecology, Inc., Attn: Diane Kitko, Post Office Box 23263, Baltimore, MD 21203-9990, Telephone: 1-(301) 955-7822.

Why settle for less activit

Don't Compromise



Copyright © 1987 by Merck & Co., Inc.

*Fortaz is a registered trademark of Glaxo Inc.
**Cefobid is a registered trademark of Pfizer Inc.
†Claforan is a registered trademark of Roussel-Uclaf.
††Cefizox is a registered trademark of Fujisawa SmithKline Corporation.

gainst the *B. fragilis* group?

94%
Mefoxin
active against 94% of isolates

the facts *still* favor Mefoxin® IV/IM (Cefoxitin Sodium | MSD)

The *Bacteroides fragilis* group includes *B. fragilis, B. distasonis, B. ovatus, B. thetaiotaomicron, B. vulgatus.*

The percentages displayed are a weighted average of the susceptibilities reported for these organisms at an MIC breakpoint of 16 mcg/mL by the Antibiotic MIC Susceptibility Reports, National Summary, in *The Bacteriologic Report: Volume I 1986,* Totowa, NJ, BAC-DATA® Medical Information Systems, Inc. This average was weighted by the frequency of isolation of each organism.

NOTE: *In vitro* activity does not necessarily imply *in vivo* effectiveness.

MEFOXIN is contraindicated in patients who have shown hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

MEFOXIN is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Streptococcus faecalis*) and many strains of *Enterobacter cloacae.* Methicillin-resistant staphylococci are almost uniformly resistant to MEFOXIN.

MSD
MERCK SHARP & DOHME

For a Brief Summary of Prescribing Information, please see following page.

Mefoxin® IV/IM (Cefoxitin Sodium | MSD)

Indications and Usage: *Treatment*—Serious infections caused by susceptible strains of the designated microorganisms in the following diseases:

LOWER RESPIRATORY TRACT INFECTIONS, including pneumonia and lung abscess, caused by *Streptococcus pneumoniae* (formerly *Diplococcus pneumoniae*), other streptococci (excluding enterococci, e.g., *Strep. faecalis*), *Staphylococcus aureus* (penicillinase and non-penicillinase producing), *Escherichia coli*, *Klebsiella* species, *Hemophilus influenzae*, and *Bacteroides* species.

GENITOURINARY INFECTIONS. Urinary tract infections caused by *E. coli*, *Klebsiella* species, *Proteus mirabilis*, indole-positive *Proteus* (i.e., *P. morganii*, *P. rettgeri*, and *P. vulgaris*), and *Providencia* species. Uncomplicated gonorrhea due to *Neisseria gonorrhoeae* (penicillinase and non-penicillinase producing).

INTRA-ABDOMINAL INFECTIONS, including peritonitis and intra-abdominal abscess, caused by *E. coli*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ and *Clostridium* species.

GYNECOLOGICAL INFECTIONS, including endometritis, pelvic cellulitis, and pelvic inflammatory disease, caused by *E. coli*, *N. gonorrhoeae* (penicillinase and non-penicillinase producing), *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and group B streptococci.

SEPTICEMIA caused by *Strep. pneumoniae* (formerly *D. pneumoniae*), *Staph. aureus* (penicillinase and non-penicillinase producing), *E. coli*, *Klebsiella* species, and *Bacteroides* species including the *B. fragilis* group.§

BONE AND JOINT INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing).

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis*, streptococci (excluding enterococci, e.g., *Strep. faecalis*), *E. coli*, *P. mirabilis*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae*. Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.

Contraindications: Previous hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

Warnings: BEFORE THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOXITIN, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. GIVE WITH CAUTION TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. IF AN ALLERGIC REACTION TO CEFOXITIN OCCURS, DISCONTINUE THE DRUG. SERIOUS HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

Pseudomembranous colitis, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use. Broad-spectrum antibiotics alter normal flora of colon and may permit overgrowth of clostridia; a toxin produced by *Clostridium difficile* is a primary cause of antibiotic-associated colitis. Mild cases may respond to drug discontinuance alone; in more severe cases, management may include sigmoidoscopy, appropriate bacteriological studies, fluid, electrolyte and protein supplementation, and use of a drug such as oral vancomycin; isolation of the patient may be advisable. Other causes of colitis should also be considered.

Precautions: *General*—Total daily dose should be reduced in patients with reduced urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur from usual doses. Prescribe with caution in patients with a history of gastrointestinal disease, particularly colitis. Prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures.

Drug Interactions—Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.

Drug/Laboratory Test Interactions—High concentrations (>100 mcg/mL) may interfere with measurement of serum and urine creatinine levels by the Jaffé reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. High concentrations may interfere with measurement of urinary 17-hydroxy-corticosteroids by the Porter-Silber reaction and produce false increases of modest degree in levels reported. A false-positive reaction for glucose in urine has been observed with CLINITEST ‖ reagent tablets.

Carcinogenesis, Mutagenesis, Fertility Impairment—No long-term animal study has been performed on carcinogenic or mutagenic potential. Rat studies at approximately three times maximum recommended human dosage revealed no effects on fertility or mating ability.

Pregnancy Category B—Reproduction studies in rats and mice did not reveal teratogenic or fetal toxic effects, although fetal weights were slightly decreased. In rabbits, cefoxitin was associated with a high incidence of abortion and maternal death, neither considered teratogenic. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers—Excreted in human milk. Exercise caution.

Pediatric Use—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.

Adverse Reactions: The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently. *Local Reactions*—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. *Allergic Reactions*—Rash (including exfoliative dermatitis), pruritus, eosinophilia, fever, and other allergic reactions including anaphylaxis. *Cardiovascular*—Hypotension. *Gastrointestinal*—Diarrhea, including documented pseudomembranous colitis during or after treatment, and, rarely, nausea and vomiting. *Blood*—Eosinophilia, leukopenia including granulocytopenia, neutropenia, anemia, including hemolytic anemia, thrombocytopenia, and bone marrow depression. A positive direct Coombs test may develop in some individuals, especially those with azotemia. *Liver Function*—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase. *Renal Function*—Elevations in serum creatinine and/or blood urea nitrogen levels and, rarely, acute renal failure.

Note: In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

How Supplied: Sterile cefoxitin sodium in vials and infusion bottles containing 1 gram or 2 grams cefoxitin equivalent and in 10-gram bulk bottles.

§ *B. fragilis*, *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, *B. vulgatus*
‖ Registered trademark of Ames Company, Division of Miles Laboratories, Inc.

For more detailed information, consult your MSD Representative or see Prescribing Information. Merck Sharp & Dohme, Division of Merck & Co., INC., West Point, PA 19486 J6MF49(120)

MSD
MERCK SHARP & DOHME

SEVENTH ANNUAL MEETING

AMERICAN SOCIETY FOR LASER MEDICINE AND SURGERY

APRIL 11 - 13, 1987
SAN FRANCISCO, CALIFORNIA

Scientific specialty sessions including the latest developments in laser applications in gynecology.

APRIL 10, 1987
INTRODUCTION TO LASER BIOPHYSICS

Sponored by:
American Society for Laser Medicine and Surgery, Inc.
813 Second Street Suite 200
Wausau, Wisconsin 54401
(715) 845-9283

PERSONALIZED LIBRARY CASES

Protect your personal copies of AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY in custom made, titled library cases.

Constructed of durable, reinforced binder's board covered in a rich simulated leather, each is designed to hold an entire year's issues. The Journal name is stamped in gold leaf, making the cases attractive additions to any decor, whether on your bookshelf, desk or any location in your home or office.

Only $7.95 each: 3 for $21.95; 6 for $39.95, and $1 per unit postage and handling ($2.50 per unit outside the U.S.A.). U.S. funds only.

To charge your order using American Express, Visa, MasterCard or Diners Club, call toll-free: 1-800-972-5858.

Please send me _____ library cases for AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY at $7.95 each: 3 for $21.95; 6 for $39.95, and $1 per unit postage and handling ($2.50 per unit outside the U.S.A.).

Name _____ (please print legibly or type)

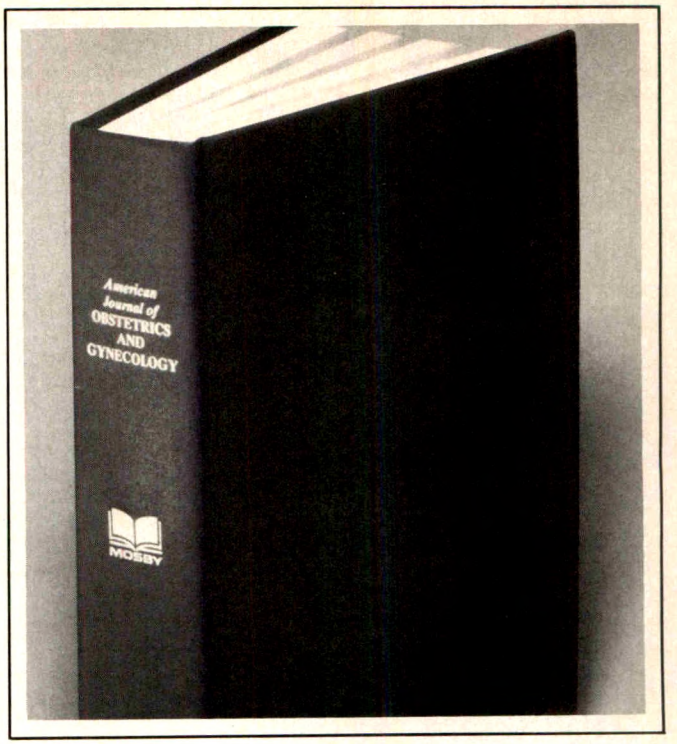
Address _____ (no P.O. boxes, please)

City/State/Zip _____

PA residents add 6% sales tax

Jesse Jones Industries
499 East Erie Avenue
Philadelphia, PA 19134
Department Code: Mosby

Here's what MOSBY'S BOUND VOLUMES can add to your practice!



MOSBY
TIMES MIRROR

Handsomely bound in durable buckram with rich gold stamping, bound journal reference volumes are important to every successful practice.

- gives quick and easy access to important journal articles
- eliminates problems with missing issues or pages
- makes your regular subscription a "working" copy to mark up, tear from, or file
- offers an inexpensive and easy way to guarantee bound reference volumes—prices include shipping

You Must Order Now!
Bound volumes are made up from over-run copies of each journal issue. You never worry about sending out your personal copies. But over-run quantities are limited. We need your order today! Bound volumes are only available to current subscribers and may not be ordered in place of a regular journal subscription. Payment must accompany your order.

Credit Card Orders In The U.S. Call FREE:
1-800-325-4177, ext. 351
(In Missouri, call collect: (314) 872-8370, ext. 351)

YES! Send me the 1987 bound volumes of:

☐ ***AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY***

Jan.-June, 1987 (Vol. 156, No. 1-6)
July-Dec., 1987 (Vol. 157, No. 1-6)
Total 1987 Price, Two Volume Set

USA	**International**
$51.00	$74.00

All orders must include payment. Bound volumes are non-returnable.

My check for $_____ is enclosed.

☐ VISA ☐ MasterCard

Card No._____

Exp. date_____

Name_____
(Please Print)

Address_____

_____

City_____

State_____ Zip_____

Clip and return coupon to: The C.V. Mosby Company, Attn: Bound Volumes, 11830 Westline Industrial Dr., St. Louis, MO 63146. Outside the U.S. please pay by check drawn on a U.S. bank in U.S. dollars or use credit card. **ABS-127**

TRI-LEVLEN™

Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

A LOW-DOSE TRIPHASIC OC WITH THE CONFIDENCE THAT COMES FROM EXPERIENCE



*Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. The physician should remain alert to the earliest manifestations of any symptom of serious disease and discontinue oral contraceptive therapy when appropriate. These d represent only the results of selected studies. Therefore, before prescribing, the physician should read and remain alert to all data found in the complete prescribing information for the product. Please also see brief summary of prescribing information on the last page of advertisement.

A formulation with the lowest total hormonal dose in a combination OC

Phased doses of both levonorgestrel and ethinyl estradiol

Permits a decrease in total steroid dose without compromising effectiveness.

Minimal impact on the following metabolic and hemodynamic parameters*

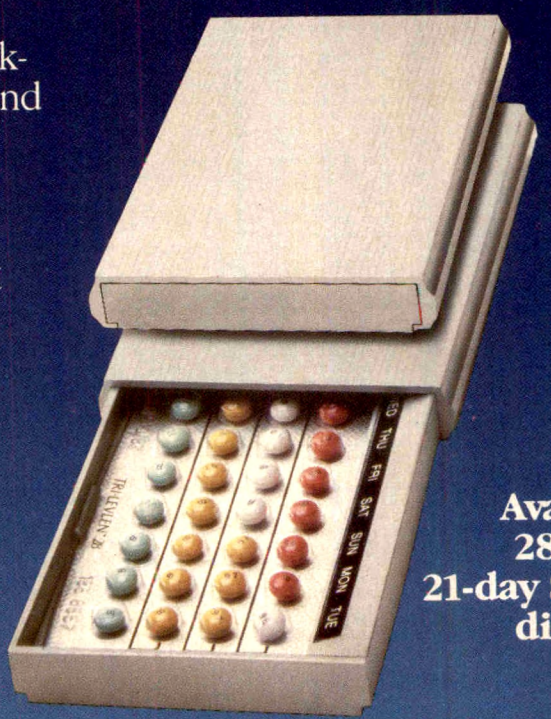
Lipids;[2,3] carbohydrate metabolism[2,3] and blood pressure[1] were not significantly affected in certain specific studies.

Low incidence of breakthrough bleeding

A low 5.7% overall incidence of breakthrough bleeding based on 77 U.S. and U.K. studies involving 3,546 women (35,036 total cycles).[1]

Simple first dose/first day start

The first dose is taken on the first day of menstruation. No need to count days or wait for Sunday starts.



A formulation with the confidence of 170 million cycles of experience worldwide

Available in 28-day and 21-day Slidecase™ dispensers

TRI·LEVLEN™28 TRI·LEVLEN™21

BERLEX™ **Dedicated to innovation in OB/GYN medicine**

TRI·LEVLEN™ 28
TRI·LEVLEN™ 21

Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

BRIEF SUMMARY

Tri-Levlen™—6 brown tablets, each containing 0.050 mg of levonorgestrel (d(-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxygon-4-en-3-one), a totally synthetic progestogen, and 0.030 mg of ethinyl estradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3, 17-diol); 5 white tablets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol; 10 light-yellow tablets, each containing 0.125 mg levonorgestrel and 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day triphasic regimen).

Indications and Usage—Tri-Levlen Tablets are indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OCs) as a method of contraception.

Contraindications—OCs should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during the use of OCs or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thrombophlebitis and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to-six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions:* There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.

4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, an increased risk was apparent between 6 and 12 months of use.

7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. Three studies have been performed with the Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alterations in lipid metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.

9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.

10. *Bleeding Irregularities:* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding:* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See "Warnings" section for information on the carcinogenic potential of OCs.

Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."

Nursing Mothers—See "Warnings."

Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted.: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is first taken later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information and package insert.

References: 1. Data submitted to FDA. **2.** Skouby SO, Kühl C, Molsted-Pedersen L, et al: *Am J Obstet Gynecol* 1985; 153:495-500. **3.** Vermeulen A, Thiery M: *Contraception* 1982;26:505-513.

BERLEX™
Dedicated to innovation in OB/GYN medicine

Berlex Laboratories, Inc., Wayne, New Jersey 07470

© 1986 Berlex Laboratories, Inc. All rights reserved.

430-27

Chorioamnionitis: Its association with pregnancy outcome and microbial infection

P. A. Quinn, Ph.D., J. Butany, M.D., J. Taylor, M.D., and W. Hannah, M.D.

Toronto, Ontario, Canada

In a study of 33 cases of perinatal death, chorioamnionitis was observed in 57.6% compared with 5% of 20 control cases ($p < 0.001$) and in 70.8% of cases with no morphologic cause compared with 22.2% of cases with a defined cause of death ($p < 0.01$). Chorioamnionitis was significantly associated with previous gestations ($p < 0.01$), prolonged rupture of the membranes ($p < 0.001$), prematurity (20 to 27 weeks' gestation) ($p < 0.001$), and low birth weight (≤1000 gm) ($p < 0.001$) but not with elevated maternal white blood cell count or pyrexia. Overall, in patients with chorioamnionitis, the perinatal death rate was higher ($p < 0.01$); more stillbirths occurred compared with early neonatal deaths ($p < 0.05$), and there was a higher incidence of deaths with no defined cause ($p < 0.01$) compared with cases without chorioamnionitis. *Ureaplasma urealyticum* or pathogenic bacteria were isolated more frequently from villous tissue of placentas from cases with chorioamnionitis ($p < 0.01$) but not *Mycoplasma hominis, Chlamydia trachomatis,* or viruses. Furthermore, there was a higher prevalence of both elevated fetal antibody titer to *U. urealyticum* ($p < 0.025$) and fetal titers fourfold above maternal titers ($p < 0.05$) in cases with chorioamnionitis. The antibody responses and presence of microorganisms suggest that chorioamnionitis is associated with intrauterine infection and an associated increase in perinatal morbidity and mortality. (Am J Obstet Gynecol 1987;156:379-87.)

Key words: Chorioamnionitis, perinatal death, pregnancy outcome, microbial infection, *Ureaplasma urealyticum*

It has been believed that the intact placental membranes are a good barrier to ascending infections but this may be only partly true.[1] Depending on the study, reports indicate that between 5% and 40% of placentas show acute chorioamnionitis.[1, 2] Although the evidence for this has been equivocal, as microorganisms are isolated only in about 40% of cases,[2, 3] chorioamnionitis due to microbial infection is considered a significant cause of perinatal morbidity and mortality.[4] It is possible that the inability to culture mycoplasmas, viruses, chlamydia, anaerobes, and even some aerobes is due to inadequate sampling or improper culture techniques required for the isolation of these more fastidious microorganisms.

Often in examining a placenta histologically, one notes the presence of acute inflammatory cells in the amnion, chorion, or umbilical cord. The significance of these polymorphonuclear leukocytes is questioned, that is, are they related to premature rupture of the membranes or microbial infection, or are they nonspecific? It has also been said that the presence of polymorphonuclear leukocytes is common in the first and second trimesters of pregnancy and that their presence is nonspecific and not associated with pathogenic microorganisms.[3] However, the presence of polymorphonuclear leukocytes and acute chorioamnionitis may be due to different reasons. Our studies of perinatal death have shown a significant incidence of acute inflammation in the placenta and/or fetal lung in fatal cases compared with normal controls.[5] Second, this inflammation and the corresponding detection of bacteria and *Ureaplasma urealyticum* occurred primarily in fatal cases with no other recognized cause of death.

Previous studies examined either the placenta only[6-8] or the neonate.[9] In this study the placenta, fetus-neonate, and maternal and fetal blood were examined extensively. We undertook this study to determine whether there is an association between chorioamnionitis, microbial infection, and perinatal death on the basis of both perinatal deaths and normal term deliveries. Microbiologic and serologic tests were used to detect bacteria, mycoplasmas, viruses, and chlamydia.

From the Department of Bacteriology, The Hospital for Sick Children, and the Departments of Pathology and Obstetrics and Gynecology, Toronto General Hospital, Women's College Hospital, and the University of Toronto.

This project was supported by Health and Welfare Canada Grant No. 6606-2140-53 and The Hospital for Sick Children Foundation Grant No. 84-46.

Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.

Reprint requests: J. Butany, M.D., Department of Pathology, Eaton Building, 4-305, Toronto General Hospital, 200 Elizabeth St., Toronto, Ontario, Canada M5G 1L7.

Material and methods

Population studied. This prospective study was conducted at two University of Toronto teaching hospitals. All patients entered into the study were admitted either to Women's College Hospital (a hospital with a large high-risk pregnancy service and a regional perinatal unit) or to Toronto General Hospital, for delivery during the period June 1982 to February 1984. All patients were covered by a provincial health insurance plan. Cases of perinatal death included fetal death in utero after 20 weeks' gestation, or death of the neonate within 48 hours after delivery. The study included all consecutive cases with consent in which autopsy was performed on week days. The placenta was examined in detail and a complete autopsy performed. The majority of cases were cared for by obstetricians at the respective hospitals with details of the pregnancy easily obtained. In a minority of cases the patients were transferred to the Regional High-Risk Pregnancy Service (Women's College Hospital) just before delivery, and complete clinical histories could not be obtained.

The control group consisted of placentas taken from 20 random cases of normal term deliveries, occurring on weekdays, to accommodate laboratory services. The criteria for inclusion of cases as normal controls consisted of a term delivery with rupture of the membranes of <24 hours and no history of reproductive failure or problems in the current pregnancy. All clinical histories were reviewed to determine the presence of maternal fever, to evaluate the white blood cell count, and to check the duration of ruptured membranes.

For certain analyses the cases of perinatal death were divided into two groups: In group 1 were those with "anomalies" acceptable as a cause of death, such as multiple congenital abnormalities (Turner's syndrome) or severe hydrops fetalis with acute polyhydramnios, plus cases with a questionable anatomic cause of death, such as congenital abnormalities complicated by prematurity. There were no cases in which abruptio placentae was considered to be a significant factor. In group 2 were those with "no anomalies" (morphologic or anatomical) that would be considered the cause of death.

To examine the association of chorioamnionitis with pregnancy outcome and infection, the 53 cases were grouped and compared on the basis of the presence or absence of chorioamnionitis, as defined below.

Specimen collection. In the control group, samples of maternal and umbilical cord blood were collected at the time of delivery. A 3 cm cube of placenta was taken in the delivery suite with aseptic precautions and placed in a sterile container. All samples were taken to the microbiology laboratory as soon as possible and set up immediately. The placenta was sent to the department of pathology for histologic examination, and the fetus or neonate was submitted for autopsy. A maternal blood sample was requested. At autopsy, blood samples drawn from the fetus, placental tissue, and other postmortem specimens were submitted for microbiologic studies. A few cases were omitted when the fetus was severely macerated and the tissues badly autolyzed. If the fetus was of very low birth weight and the fetal blood sample could not be obtained or was hemolyzed, the case could not be adequately examined.

Pathologic examination. For the gross examination each placenta was weighed after "standardization," that is, the placental cake along with a 2 cm rim of membranes and 5 cm stump of umbilical cord. This was done to avoid differences in weight due to variations in cord lengths. The placenta, cord, and membranes were examined in detail. A comprehensive autopsy was performed in each case beginning with an external examination and followed by examination of the viscera.

Histologic examination. For histologic examination of the placenta, five blocks were taken from each placenta, including one strip of free membranes and umbilical cord and four full-thickness blocks of the placental cake. Histologic sections were stained with hematoxylin and eosin, and when evidence of inflammation was seen, sections were stained for bacteria (Gram and Gomori methenamine silver stains).

The histologic criterion used for acute inflammation of the placenta (chorioamnionitis) was the presence of accumulations of polymorphonuclear leukocytes extending through the fetal membranes and/or the umbilical cord.[10] Polymorphonuclear leukocytes were generally seen in the chorionic plate, under the amnion, and at times in the intervillous space under the chorionic plate.[10] All histologic sections of the placenta were examined by one of us without knowledge of the microbiologic findings (J. B.).

Microbiologic methods. The placental tissue was surface sterilized by burning with alcohol and cut open through the chorionic surface. A block of villous tissue was removed from the inside for culture to avoid surface contamination and obtain an indication of microorganisms actually present in the placental substance. For bacteria, this piece of placenta was minced lightly; a Gram stain was done, and the specimen was inoculated on media to isolate aerobic and anaerobic bacteria and fungi as previously described.[5] For mycoplasmas, the tissue specimens were lightly minced, not ground, and inoculated into two broth and agar culture systems for the isolation of large colony mycoplasmas and *U. urealyticum*.[5] Three tenfold serial dilutions were made for broth cultures. All negative cultures were blind passaged on days 3 and 7 and incubated for 21 days. All medium components and each medium batch were routinely quality controlled for adequate growth-promoting capacity. Tissues were frozen at −70° C in transport media containing gentamicin (10 μg/ml),

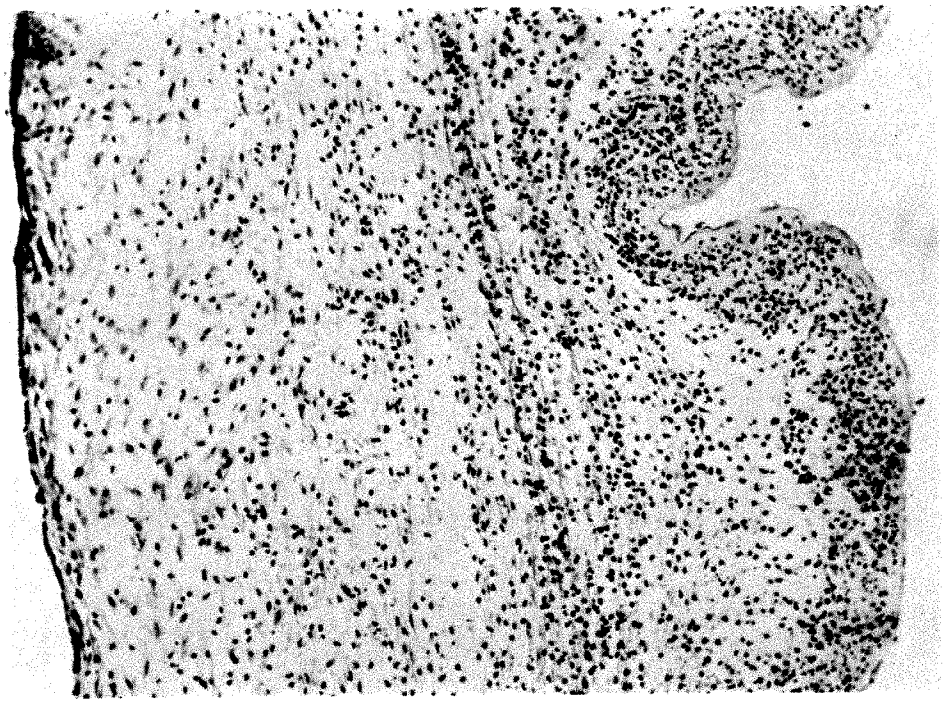


Fig. 1. Acute chorioamnionitis. Histologic section of the fetal surface of the placenta showing acute chorioamnionitis. There is an infiltration of the amnion and the chorion by polymorphonuclear leukocytes. (Original magnification ×100.)

streptomycin (50 μg/ml), and nystatin (25 μg/ml) for the isolation of chlamydia in cycloheximide-treated HeLa cells.[11] The tissues were frozen at −70° C and set up for the isolation of viruses in skin fibroblasts, green monkey kidney, and HeLa 293 cells.[11]

Serologic methods. A modified metabolic inhibition test[12] was used to measure the patient's antibody response to eight serotypes of *U. urealyticum* and *Mycoplasma hominis* strain PG-21. Any sera showing nonspecific inhibition to all serotypes was excluded. Antibody titers to *Chlamydia trachomatis* were detected by the microimmunofluorescence test.[13] The hemagglutination test was used for rubella, the complement fixation test for cytomegalovirus and herpes simplex virus, and the neutralization test for enterovirus and coxsackie virus B.[11] For *U. urealyticum* an antibody titer of ≥1:32, for *M. hominis* a titer of ≥1:8, and for chlamydia and viruses a titer ≥1:64 was scored as elevated. Rubella-positive titers were excluded. When limited sera were available, priority was given to the mycoplasma titers.

Statistical methods. Comparisons were made between chorioamnionitis-positive versus chorioamnionitis-negative placentas. In each test of significance, the χ^2 test, corrected for continuity, was used. For groups with small numbers, Fisher's exact test was used and performed at a 5% level of significance in both tails of the distribution. For borderline values, the exact p value is given.

Results

Chorioamnionitis in cases of perinatal death versus controls. Of the 53 placentas examined, chorioamnionitis was found in 20 (Figs. 1 and 2). Since there was greater evidence of infection in cases of perinatal death,[5] the significance of placental chorioamnionitis was assessed. The data presented in Table I clearly show a higher incidence of 57.6% in cases with chorioamnionitis in the perinatal death group compared with 5% in the control group ($p < 0.001$). The perinatal deaths were then divided into two groups, those with "anomalies" that were a possible cause of death and those with "no anomalies" or no morphologic cause of death. Among those with no anomalies, chorioamnionitis was observed in 74.9% of cases compared with 12.5% of cases with no anomalies ($p < 0.01$).

Among the cases of perinatal death examined for the presence of microorganisms, *U. urealyticum* was isolated from 57.9% and pathogenic bacteria from 26.1% of placentas compared with 10% and 0%, respectively, in control placentas ($p < 0.01$ and $p < 0.05$). There were no significant isolations of *M. hominis*, viruses, or *C. trachomatis*. When the perinatal deaths were assessed, most of the *U. urealyticum* isolations came from placentas in the group with no congenital anomalies ($p < 0.05$). The isolation of pathogenic bacteria and *M. hominis* occurred more often in the same group but was not statistically significant.

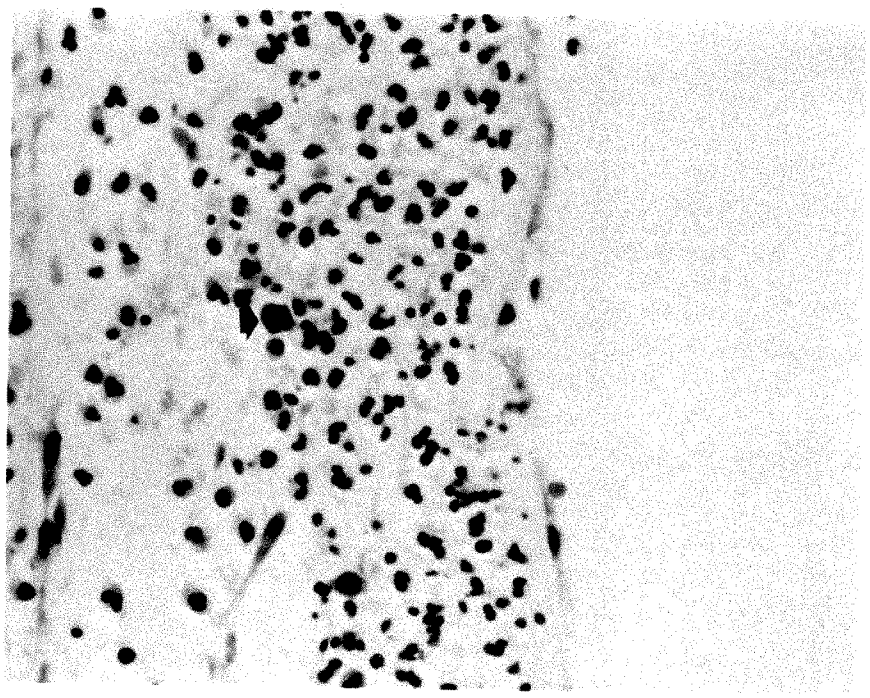


Fig. 2. Higher magnification of Fig. 1 shows that most of the inflammatory cells are polymorphonuclear leukocytes with a few macrophages *(arrowhead)*. (Original magnification ×400.)

Table I. Incidence of microorganisms isolated from the placenta: Perinatal deaths versus controls

Status	*Chorioamnionitis positive*	*Pathogenic bacteria*	*Mycoplasmas*		*Viruses*	*Chlamydia*
			U. urealyticum	M. hominis		
Control	1/20	0/20	2/20	0/20	ND	0/20
Perinatal death	19/33	6/23	10/23	1/23	0/10*	0/9*
p Value	<0.001	<0.05	<0.01	NS	–	NS
Cause of death						
Anomalies	2/9	1/5	0/5	0/5	0/4	0/3
No anomalies	17/24	5/18	10/18	1/18	0/6	0/6
p Value	<0.01	NS	<0.05	NS	NS	NS

ND = Not determined.

*In four cases the tissues were toxic to the cell cultures.

These observations support the concept that chorioamnionitis associated with *U. urealyticum* and pathogenic bacteria are important factors in perinatal death, particularly among the 72.7% of cases with no other morphologic cause of death. In addition, when the surface of the placenta is carefully decontaminated, few bacteria are isolated, and only 10% of control placentas from normal term delivery with no previous history of reproductive failure have *U. urealyticum* in our population.

Correlation of chorioamnionitis with obstetric history. The obstetric history of patients with and without chorioamnionitis is outlined in Table II. Among the 53 patients studied, 20 had chorioamnionitis and 33 did not. Chorioamnionitis was observed significantly more often in women with previous pregnancies than in primigravid patients. There was no difference in the number of previous losses or previous living children with this size population.

Correlation of chorioamnionitis with gestational age and birth weight. The distribution of gestational age and birth weight is examined in Table III. There was a significantly higher number of very premature fetuses (20 to 27 weeks' gestation) among women with chorioamnionitis ($p < 0.001$) and correspondingly with birth weights <1000 gm ($p < 0.001$) than among the women with normal placentas. In the chorioamnionitis-negative group, 66% of the fetuses-neonates were >38 weeks of gestational age, and 69.6% had birth weights ≥2500 gm. Since all the control infants and many of the stillborn infants with congenital anomalies were delivered near term and were mainly chorioamnionitis

Table II. Correlation of chorioamnionitis with obstetric history

			Multigravid women		
	No.	*Primigravid women*	*No.*	*No. of previous losses*	*No. of previous living children*
Chorioamnionitis positive	20	3	17	11	6
Chorioamnionitis negative	33	18	15	7	2
p Value			<0.01	NS	NS

Table III. Correlation of chorioamnionitis with gestational age and birth weight

		Gestational age				*Birth weight*		
	No. of cases	*20-27 wk*	*28-31 wk*	*32-37 wk*	*≥38 wk*	*≤1000 gm*	*1001-2499 gm*	*≥2500 gm*
Chorioamnionitis positive	20	14	2	3	1	16	2	2
Chorioamnionitis negative	33	5	4	2	22	4	6	23
p Value		<0.001	NS	NS		<0.001	NS	

Table IV. Correlation between chorioamnionitis and clinical parameters

	Elevated white blood cell count		*Pyrexia*		*Prolonged rupture of membranes (≥24 hr)*
	≥11 × 10⁹/L	*≥13 × 10⁹/L*	*≥37.5° C*	*≥38.0° C*	
Chorioamnionitis positive	12/19	7/19	5/20	4/20	10/19
Chorioamnionitis negative	15/31	5/31	9/33	3/33	1/32
p Value	NS	NS	NS	NS	<0.001

negative, the higher gestational ages and birth weights would be expected.

Correlation of chorioamnionitis with clinical parameters. Clinical evidence of chorioamnionitis may include elevated white blood cell count and pyrexia. In this population there was no significant elevation in white blood cell count, even at ≥13 × 10^9/dl, or pyrexia among chorioamnionitis-positive compared with chorioamnionitis-negative women in Table IV. However, chorioamnionitis did occur significantly more often in women with rupture of the membranes of ≥24 hours ($p = 0.00013$). Thus, in this study, elevated white blood cell count and pyrexia were not significant indicators of underlying chorioamnionitis. On the other hand, in 10 patients with prolonged rupture of the membranes, five had white blood cell counts of ≥13 × 10^9/dl, and three had pyrexia. There seems to be more of an association with clinical evidence of infection in bacteria-positive cases than in mycoplasma-positive cases.[5] At most, 26% of women in the group with chorioamnionitis and none of the control subjects had evidence of "clinical" infection before delivery.

Correlation of chorioamnionitis with pregnancy outcome. The population studied included fetal-neonatal deaths and normal term neonates. When the pregnancy outcome is examined in relation to chorioamnionitis, one would expect an even distribution of outcomes; that is, neonatal deaths or congenital anomalies would occur equally in each group. This was the case among the chorioamnionitis-negative group, as shown in Table V, the number that survived versus the number that died and the number of neonatal deaths versus the number of stillbirths being about equal. However, among the chorioamnionitis-positive cases significantly more deaths occurred ($p < 0.01$), and more were associated with stillbirth ($p < 0.05$) than with early neonatal death.

Correlation of chorioamnionitis with isolation of microorgansims. To determine the correlation between chorioamnionitis and the presence of microorganisms, a wide spectrum of cultures was done on the villous tissue to obtain a true indication of microorganisms actually in the placental tissue. Two approaches were used to address the question; higher virulent strains only and all bacteria isolated were used in the analyses. As outlined in Table VI, *U. urealyticum* and/or pathogenic bacteria were isolated more often from the placenta in chorioamnionitis-positive cases than in negative cases ($p < 0.01$). Analysis of a greater number of cases is required to clarify the status of each alone.

Table V. Correlation between chorioamnionitis and pregnancy outcome

	No. surviving	*Died*			*Cause of death*	
		No.	*Neonatal death*	*Stillbirth*	*With anomalies*	*Without anomalies*
Chorioamnionitis positive (n = 20)	1	19	2	17	2	17
Chorioamnionitis negative (n = 33)	19	14	7	7	7	7
p Value		<0.01	NS	<0.05	NS	<0.01

Table VI. Correlation between chorioamnionitis and the isolation of microorganisms

	U. urealyticum	*Bacteria*		*Viruses*	*Chlamydia*	*Bacteria or ureaplasma*
		Pathogenic	*Any*			
Chorioamnionitis positive	7/14	4/14	4/14	0/4	0/5	10/14 (71.4%)
Chorioamnionitis negative	5/29	1/29	7/29	0/6	0/24	8/29 (27.6%)
p Value	0.064	0.065	NS	NS	NS	<0.01

Table VII. Correlation between chorioamnionitis and elevated antibody responses

	Elevated antibody titers to								
	U. urealyticum*			M. hominis†		*Viruses‡*		*Chlamydia‡*	
	Fetus	*Fetus 4× above mother*	*Mother*	*Fetus*	*Mother*	*Fetus*	*Mother*	*Fetus*	*Mother*
Chorioamnionitis positive	9/12	7/7	8/14	1/12	0/14	0/5	3/11	0/6	0/11
Chorioamnionitis negative	8/29	12/25	9/26	3/28	2/26	5/20	5/20	3/20	2/20
p Value	<0.025	<0.05	NS	NS	NS	NS	NS	NS	NS

*Elevated titer taken as ≥1:32 to one or more *U. urealyticum* serotypes 1 to 8.
†Elevated titer taken as ≥1:8 to the standard strain PG-21.
‡Elevated titer taken as ≥1:64 for viruses and chlamydia.

Among the five cases considered to have pathogenic bacteria, one had β-hemolytic streptococci, Lancefield group B, alone, one had *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Bacteroides melaninogenicus;* and one each had *Citrobacter freundii* and *B. melaninogenicus. M. hominis* was isolated only once from a control patient. Overall either pathogenic bacteria and/or *U. urealyticum* isolated from 71.4% of chorioamnionitis-positive cases compared with 27.6% of chorioamnionitis-negative cases ($p < 0.01$). The combined presence of these organisms was previously documented.[5] Viruses or chlamydia were not isolated from the placenta in this study. In our experience, *C. trachomatis* has been isolated only once from 250 placentas cultured. This occurred in a term pregnancy in a patient with successful management of premature onset of labor (unpublished observations). These observations suggest that there is a correlation between the presence of inflammation in the placenta and the isolation of *U. urealyticum* and pathogenic bacteria.

Among the chorioamnionitis-negative group there were six additional cases in which strains considered to be of low virulence were isolated. These included one with *Streptococcus faecalis, Staphylococcus epidermidis,* and *Candida albicans,* one with *S. epidermidis* and enteric streptococci, and ones with *Bacteroides vulgaris, Clostridium* sp., *Streptococcus viridans,* and *Escherichia coli.* In all cases, these yielded only a few colonies, compared with a heavy growth of the more pathogenic stains. If the presence of all bacteria is included, there is no significant difference in the isolation rate, 28.6% versus 24.1%, between chorioamnionitis-positive and -negative cases. Perhaps the specific species, the actual concentration of organism, the location of bacteria, and the combination of bacteria with *U. urealyticum* are the important factors in understanding the association of chorioamnionitis with microorganisms.

Correlation of chorioamnionitis with elevated antibody titers. Another aspect of examining fetal-maternal infection was to assess the antibody responses in cases with and without chorioamnionitis. As shown in Table VII, the prevalence of elevated antibody response to *U. urealyticum* at a titer of ≥1:32 was 75.0% in fetuses from pregnancies with chorioamnionitis com-

pared with 27.6% in control pregnancies ($p < 0.025$). The prevalence of elevated ureaplasma antibody in the mothers with chorioamnionitis was 57.1% compared with 34.6% in those without (NS). Since maternal immunoglobulin G could cross to the fetus, the prevalence of a fourfold higher fetal titer above the maternal titer, suggestive of fetal immunoglobulin M response, was determined. Among chorioamnionitis-positive cases with complete serologic evaluation, the fetus had a four times higher titer than the mother in 100% of the cases compared with 48% in the chorioamnionitis-negative cases ($p < 0.05$). These observations suggest that in cases of chorioamnionitis, the fetus produces antibody indicative of intrauterine infection with *U. urealyticum*. In cord sera from normal term gestations, only 6.5% of neonates have titers $\geq 1:32$ and 2.2% have titers fourfold above maternal levels.[12]

An antibody response to *M. hominis* with the use of the metabolic inhibition test and strain PG-21 was detected in five cases, all controls. This correlates with the low isolation rate of *M. hominis* in this study. On the other hand, the use of strain PG-21 may not be adequate to detect antibody responses to all serotypes (Quinn PA, et al., paper presented at the Sixth International Congress of the International Organization for Mycoplasmology, Birmingham, Alabama, August 22-31, 1986). An elevated fetal and maternal antibody response to viruses and chlamydia ($\geq 1:64$) occurred more often in the chorioamnionitis-negative group than in the positive group.

Comment

Whether the inflammatory response observed in the placenta is due to infection is a much debated point. Infection can occur through intact membranes with monilia,[3] *U. urealyticum*, and *M. hominis*[14] resulting in clinically silent chronic intrauterine infection characterized by an intense inflammatory response. Infection can also occur with rupture of the membranes as extensively documented.[3]

In determining the cause of perinatal death, no morphologic cause can be documented at autopsy of the fetus in nearly two thirds of cases.[4, 5, 15] Naeye et al.[10] reported that acute chorioamnionitis occurred in 53% of stillbirths and early neonatal deaths. Among these chorioamnionitis-positive cases, 45 of 48 infants were born prematurely and 42 of 48 died in the perinatal period, of causes associated with prematurity and no anatomic cause of death.[19] In our previous studies, an increased incidence of inflammation in the placenta and/or fetal lung and a higher isolation rate of *U. urealyticum* were observed in perinatal deaths compared with controls, especially among those with no anatomic cause of death.[5]

In the present study, chorioamnionitis was observed in 57.6% of 33 perinatal deaths, and only 5% of normal term deliveries among women carefully documented to have no previous history of pregnancy loss or premature delivery. Furthermore, 70.8% of chorioamnionitis-positive placentas occurred among cases with no cause of death versus 22.2% of cases with other causes. Although evidence of infection is observed more often in cases with no clear-cut cause of death, it is also seen in 20% of those with other problems. Perhaps in the latter cases the infection is a contributing factor in the death of the fetus. These observations all support the concept that chorioamnionitis associated with *U. urealyticum*, alone or in combination with bacteria, is a major factor in intrauterine infections associated with perinatal morbidity and mortality.

What causes chorioamnionitis is a controversial subject because of the diversity of microorganisms isolated, often of low virulence,[4, 6, 8] and the concept that nonspecific placental inflammation may occur.[3] It has been reported that a significantly higher rate of poor outcomes occurred when genital mycoplasma, particularly *U. urealyticum*, were isolated from placentas.[8] Isolation of *U. urealyticum* has been associated with an increased incidence of chorioamnionitis, low birth weight, spontaneous abortion, and stillbirth, as well as early neonatal death and morbidity, but not prolonged rupture of the membranes.[5, 8, 14] The latter observation fits with the concept of a chronic, silent chorioamnionitis occurring before rupture of the membranes with genital mycoplasmas.[14]

Pankuch et al.,[6] in a study looking for aerobic and anaerobic bacteria and chlamydia, found positive bacterial cultures in 72% of placentas having evidence of chorioamnionitis while only 15% of placentas not having histologic evidence of chorioamnionitis had positive cultures. About 50% of their positive cultures were associated with anaerobic bacteria. In our previous report on microbial infection in perinatal deaths,[5] one of the most significant findings was that the perinatal deaths were associated with a mixed spectrum of bacteria, genital mycoplasma, and viruses. In only one of 12 bacteria-positive cases were no other organisms isolated. However in 11 of 20 mycoplasma cases no other bacteria were found. As our data and Naeye's[4] suggest, diverse microorganisms, perhaps of lower virulence but in combination with for example *U. urealyticum*, may be significant. Overall, we found that 71.4% of chorioamnionitis-positive cases had *U. urealyticum* or other pathogenic bacteria without strict anaerobic cultures being done, compared with 17.6% of the chorioamnionitis-negative cases. Thus the actual incidence of microorganisms in chorioamnionitis-positive placentas may be even higher than 72% if cultures could be done for all fastidious organisms at once.

On the other hand, in the studies of Svensson et al.,[7]

no correlation between chorioamnionitis and bacteria were found in placentas with delivery by cesarean section. The authors did not clearly set out the indications for the cesarean sections nor did they give the exact duration of pregnancy, except that in 18 cases the delivery was premature (before the thirty-seventh week). If organisms such as *U. urealyticum* are associated with spontaneous loss and prematurity, then the control population should have no previous history of problems that may be associated with infection. However, histologic chorioamnionitis did correlate with maternal or neonatal infection. In their study, the placental surface was not decontaminated. Few details were given on how *M. hominis* was isolated and no details were given for the isolation of *U. urealyticum*. This is a crucial point because mycoplasmas are not readily isolated from ground tissue commonly used to detect bacteria.[16] They reported that in no cases were *U. urealyticum* or *M. hominis* isolated and only 14% of patients had cervical cultures that were positive for ureaplasmas. This incidence appears very low since reports on female colonization with *U. urealyticum* vary from 20% to 80% of pregnant women.[16] In our studies, *U. urealyticum* is isolated from 10% of the placentas of normal deliveries among women with no previous history of pregnancy loss or prematurity[5] and 20% of any term gestations (Grundman H, Milligan J, Shennan A, et al. The association of *Ureaplasma urealyticum* with preterm birth, presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, June 23-27, 1986). When we analyzed all cases with bacteria isolated, including those with scanty growth of any organisms, we also found no association with chorioamnionitis. Perhaps the key to understanding the association of microorganisms in chorioamnionitis is to examine not only the bacterial species isolated but also their concentration and presence with other organisms such as *U. urealyticum*.

Mueller et al.,[15] in a study of 124 stillbirths or early neonatal deaths, reported that only 35% had structural physical abnormalities associated with achromosomal, single-gene, or polygenic disorders. Thus histologic examination of tissues obtained at autopsy is not necessary. In view of the high yield of chorioamnionitis in our study cases and its association with positive cultures, we feel that it definitely is of value. We agree with Naeye[4] that histopathologic analysis of autopsied tissues, particularly the placenta, can provide answers to many questions. In addition to providing a morphologic explanation for the perinatal loss, it can help to assuage the family's feeling of guilt about their role (if any) in the stillbirth. This in itself is significant when no cause of death is found in 65% to 70% of cases.[5, 15]

In such studies of human disease one cannot prove causation but only observe and accumulate supportive evidence. The isolation of organisms does not prove that they cause the pathologic lesion or fetal death. However, isolation of an organism, evidence of an inflammatory response in the placenta, and development of a fetal antibody response are strong evidence of infection in a given pregnancy, indicative of an association between the presence of infection and the stillbirth. The strongest evidence comes from the bovine model, where *Ureaplasma diversum* is also associated with reproductive failure, endometritis, and salpingitis. When *U. diversum* was experimentally inoculated into the amniotic fluid of pregnant heifers, it induced abortion and premature delivery of a calf with only respiratory disease in all cases.[17, 18] The calves exhibited patchy alveolitis similar histologically to that reported in humans.[19] These data certainly prove that in bovines, *U. diversum*, a very closely related species, causes infection in pregnancy, leading to spontaneous abortion, placentitis, and prematurity with neonatal respiratory disease.

In conclusion, our study shows that chorioamnionitis is a significant finding in cases of stillbirth. In a large number of cases it is associated with positive cultures of *U. urealyticum* and pathogenic bacteria, which often occur together.[5] Chorioamnionitis is also positively associated with a significant incidence of prematurity, low birth weight, and a poor outcome of pregnancy. On the basis of our results, we strongly recommend that in all cases of perinatal death a complete autopsy, including examination of the placenta, be done. In each case comprehensive cultures of the placenta and serologic studies on maternal and fetal blood, where possible, should be an integral part of the investigation. Only in this manner can we get a clearer picture of the pathogenesis of chorioamnionitis and develop methods to diagnose and treat such women before the pregnancy is lost.

We wish to thank the obstetricians at Women's College Hospital and Toronto General Hospital for the use of their cases. The expert technical assistance of A. Yau, H. C. S. Li, S. Mitra, and D. Askew, in the Department of Bacteriology, and the staff in the Department of Virology at The Hospital for Sick Children and the Department of Microbiology at Women's College Hospital is much appreciated.

REFERENCES

1. Hameed C, Tejani N, Verma UL, Archbald F. Silent chorioamnionitis as a cause of preterm labor refractory to tocolytic therapy. Am J Obstet Gynecol 1984;149:726.
2. Shurin PA, Alpert S, Rosner B, et al. Chorioamnionitis and colonization of the newborn infant with genital mycoplasmas. New Engl J Med 1975;293:1.
3. Blanc WA. Pathology of the placenta and cord in ascending and in haematogenous infection. In: Ciba Foundation symposium on perinatal infections. Amsterdam: Excerpta Medica, 1980, 77:17.

4. Naeye RL. The investigation of perinatal deaths. N Engl J Med 1983;309:611.
5. Quinn PA, Butany J, Chipman M, et al. A prospective study of microbial infection in stillbirths and early neonatal death. Am J Obstet Gynecol 1985;151:238.
6. Pankuch GA, Applebaum PC, Lorenz RP, et al. Placental microbiology and histology and the pathogenesis of chorioamnionitis. Obstet Gynecol 1984;64:802.
7. Svensson L, Ingemarsson I, Mardh PA. Chorioamnionitis and the isolation of microorganisms from the placenta. Obstet Gynecol 1986;67:403.
8. Kundsin RB, Driscoll SG, Monson RR, et al. Association of *Ureaplasma urealyticum* in the placenta with perinatal morbidity and mortality. N Engl J Med 1984;310:941.
9. Nakamura Y, Hosokawa Y, Hiroshi Y, et al. Primary causes of perinatal death: an autopsy study of 1000 cases in Japanese infants. Hum Pathol 1982;13:54.
10. Naeye RL, Maisel MJ, Lorenz RP, et al. The clinical significance of placental villous edema. Pediatrics 1983; 71:588.
11. Lennette EH, Schmidt NJ. Diagnostic procedures for viral rickettsial and chlamydial infections. Washington, DC: American Public Health Association, 1979.
12. Quinn PA. Evidence of an immune response to *Ureaplasma urealyticum* in perinatal morbidity and mortality. Pediatr Infect Dis 1986;5:S282.
13. Wang SP, Grayston JT. Immunologic relationship between genital TRIC, lymphogranuloma venereum, and related organisms in a new microtiter indirect immunofluorescence test. Am J Ophthalmol 1970;70:367.
14. Cassell GH, Davis RO, Waites KB, et al. Isolation of *Mycoplasma hominis* and *Ureaplasma urealyticum* from amniotic fluid at 16-20 weeks gestation: potential effect on outcome of pregnancy. Sex Transm Dis 1983;10:294.
15. Mueller RF, Sybert VP, Johnson J, et al. Evaluation of a protocol for post-mortem examination of stillbirths. N Engl J Med 1983;309:585.
16. Taylor-Robinson D, McCormack W. Mycoplasma in human genitourinary infections. In: Tully JG, Whitcomb RF, eds. The mycoplasmas. II. Human and animal mycoplasmas. New York: Academic Press, 1979:38.
17. Miller RB, Ruhnke HL, Doig BF, et al. The effects of *Ureaplasma diversum* inoculated into the amniotic cavity in cows. Theriogenology 1983;20:367.
18. Ruhnke HL, Palmer NC, Doig PA, et al. Bovine abortion and neonatal death associated with *Ureaplasma diversum*. Theriogenology 1984;21:295.
19. Quinn PA, Gillan JE, Markestad T, et al. Intrauterine infection with *Ureaplasma urealyticum* as a cause of fatal neonatal pneumonia. Pediatr Infect Dis 1985;4:538.

Massive genital and vaginal vault prolapse treated by abdominal-vaginal sacropexy with use of Marlex mesh: Review of the literature

Harold P. Drutz, M.D., and Lily S. Cha, M.D.
Toronto, Ontario, Canada

Two patients with massive procidentia and 13 patients with posthysterectomy vaginal vault prolapse underwent surgical procedures at Mount Sinai Hospital, Toronto, between May 1978 and February 1986. The standard procedure consisted of an abdominal sacropexy, with use of Marlex mesh to anchor the vaginal vault to the sacral promontory and retroperitonealization of the mesh. In 11 of the 15 patients, one or more concurrent procedures were performed at the same time. There were no intraoperative complications. One serious postoperative complication occurred, and one patient developed recurrent vault prolapse. Follow-up has been from 3 to 93 months with an average of 28 months. In 14 patients (93.3%) subjective and objective improvement was achieved. A review of the literature is presented (Am J Obstet Gynecol 1987;156:387-92.)

Key words: Vault prolapse, abdominal sacropexy, Marlex

Genital and vaginal vault prolapse is a distressing problem for the patient and the gynecologist. Besides causing discomfort with sitting, walking, and intercourse, vault prolapse interferes with bladder function and predisposes the patient to recurrent urinary tract infections and possible renal problems by ureteral obstruction.

Apart from the use of supportive pessaries, treatment is surgical. The aim in correction is to (1) restore the normal vaginal axis, (2) maintain existing vaginal length, and (3) provide permanent cure. Review of the literature reveals that there have been no less than 43

From the Gynecological Urology and Urodynamic Investigative Unit, Mount Sinai Hospital, and the Department of Obstetrics and Gynecology, University of Toronto.
Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.
Reprint requests: Dr. Harold P. Drutz, Ste. 1221, Mount Sinai Hospital, 600 University Ave., Toronto, Ontario, Canada M5G 1X5.

Table I. Patient profiles

Patient	*Age at time of operation*	*Month/year of operation*	*Operative procedure*	*Previous major gynecologic operation*
L. R.	65	May 1978	1. Right salpingo-oophorectomy 2. Burch retropubic colpourethropexy 3. Vault suspension (Marlex)	1. Vaginal hysterectomy, anterior and posterior colporrhaphy
C. R.	60	Oct 1978	1. Vault suspension (Marlex)	1. Total abdominal hysterectomy, bilateral salpingo-oophorectomy 2. Marshall-Marchetti-Krantz
L. H.	63	Nov 1978	1. Vault suspension (Marlex)	1. Total abdominal hysterectomy 2. Burch retropubic colpourethropexy 3. Colporrhaphy
E. W.	57	Jan 1979	1. Total abdominal hysterectomy, bilateral salpingo-oophorectomy 2. Partial vaginectomy 3. Vault suspension (Marlex) 4. Burch retropubic colpourethropexy	
A. P.	68	Nov 1980	1. Partial vaginectomy 2. Vault suspension, presacral peritoneum not opened (no mesh)	1. Total abdominal hysterectomy, bilateral salpingo-oophorectomy 2. Pereyra 3. Burch retropubic colpourethropexy
M. R.	59	Sept 1980	1. Vault suspension (mesh)	1. Total abdominal hysterectomy, bilateral salpingo-oophorectomy 2. Burch retropubic colpourethropexy
A. T.	49	Jan 1981	1. Total abdominal hysterectomy, bilateral salpingo-oophorectomy 2. Partial vaginectomy 3. Vault suspension (Marlex) 4. Burch retropubic colpourethropexy	
A. S.	64	Apr 1982	1. Burch retropubic colpourethropexy 2. Internal anterior repair 3. Vault suspension (Marlex)	1. Vaginal hysterectomy, anterior and posterior colporrhaphy 2. Anterior suburethral Marlex sling
L. C.	52	May 1982	1. Internal anterior repair 2. Vault suspension (Marlex)	1. Anterior and posterior colporrhaphy 2. Total abdominal hysterectomy, bilateral salpingo-oophorectomy 3. Marshall-Marchetti-Krantz
M. B.	82	Oct 1982	1. Bilateral salpingo-oophorectomy 2. Burch retropubc colpourethropexy 3. Internal anterior repair 4. Vault suspension (Marlex)	1. Vaginal hysterectomy, anterior and posterior colporrhaphy
W. E.	65	Nov 1982	1. Burch retropubic colpourethropexy 2. Vault suspension (Marlex)	1. Total abdominal hysterectomy, bilateral salpingo-oophorectomy 2. Anterior and posterior colporrhaphy 3. Marshall-Marchetti-Krantz 4. Pereyra
K. G.	54	Aug 1983	1. Vault suspension (Mersilene)	1. Vaginal hysterectomy, anterior and posterior colporrhaphy 2. Anterior and posterior colporrhaphy 3. Combined Marlex sling
B. T.	47	June 1985	1. Bilateral salpingo-oophorectomy 2. Vault suspension (Marlex)	1. Total abdominal hysterectomy, Marshall-Marchetti-Krantz 2. Combined abdominovaginal Marlex sling 3. Posterior colporrhaphy
S. C.	77	Jan 1986	1. Burch retropubic colpourethropexy 2. Internal anterior and posterior repair 3. Vault suspension (Marlex)	1. Total abdominal hysterectomy, bilateral salpingo-oophorectomy
L. G.	65	Feb 1986	1. Right salpingo-oophorectomy 2. Burch retropubic colpourethropexy 3. Vault suspension (Marlex)	1. Total abdominal hysterectomy, left salpingo-oophorectomy 2. Anterior suburethral Marlex sling

well-documented descriptions of operations for vaginal vault suspension.[1] Materials used for suspension have varied considerably: external oblique fascia, fascia lata, Mersilene, Marlex (Davol Rubber Co., Providence, Rhode Island), and Teflon meshes.

This article reviews the results of some of these operative procedures and describes one technique for correction of vaginal vault prolapse. It also demonstrates how correction of other genitourinary defects can be performed at the same time.

Parity	*Last follow-up*	*Results of last visit*
2	February 1986	Well-supported vault
5	November 1979	Grade I rectocele; well-supported vault
2	November 1982	Well-supported vault
3	November 1980	Grade I rectocele; well-supported vault
2	February 1981	Recurrent vaginal vault prolapse
4	December 1982	Well-supported vault
4	December 1983	Well-supported vault
0	February 1986	Well-supported vault
8	March 1986	Well-supported vault
4	December 1984	Well-supported vault
3	March 1983	Retroperitoneal psoas abscess, mesh removed (January 1983); well-supported vault
4	April 1986	Well-supported vault
3	March 1986	Well-supported vault
2	April 1986	Well-supported vault
2	April 1986	Well-supported vault; urge incontinence

Material and methods

Two women with massive genital prolapse and 13 women with posthysterectomy vaginal vault prolapse underwent corrective operations between May 1978 and February 1986. The average age was 61.8 years (range: 47 to 82 years) and the average parity was 3.2 (range: 0 to 8). All patients were healthy and active, and 13 of the 15 patients had undergone one or more

Table II. Use of prophylactic antibiotics

Patient	*Prophylactic antibiotics*	*Medical indication*	*Febrile morbidity*	*Complication*
L. R.	No	None	None	None
C. R.	No	None	None	None
L. H.	No	None	Yes	Pneumonia
E. W.	Cefazolin	None	Yes	Basal atelectasis
A. P.	No	None	Yes	Basal atelectasis
M. R.	No	None	None	None
A. T.	Cefoxitin	None	None	None
A. S.	Cefoxitin	None	None	None
L. C.	Vancomycin, streptomycin	Mitral valve stenosis	None	None
M. B.	No	None	Yes	Wound infection
W. E.	No	None	Yes	Retroperitoneal abscess
K. G.	Flagyl, netilmicin	Mitral valve stenosis	None	None
B. T.	Ampicillin, netilmicin	Mitral valve stenosis	None	None
S. C.	Cefoxitin	None	Yes	Basal atelectasis
L. C.	Cefoxitin	None	Yes	Basal atelectasis

Table III. Overall results at follow-up at 3 to 93 months (average, 28 months)

	No. of results	%
Recurrent vault prolapse (failure)	1	6.7
Postoperative complication	1	6.7
Minor posterior wall defect	2	13.3
Current success	14	93.3

previous gynecologic operations (Table I). In all, 30 gynecologic procedures had been performed on the 13 patients before their admission for abdominal sacropexy. The two women who had not undergone a previous gynecologic operation both had massive procidentia with uterus and vagina prolapsed well beyond the introitus, producing masses the size of watermelons. Both women had extracorporeal bladders, and one patient developed bladder calculi from chronic urinary stasis. Prior to vaginal vault suspension, both women required total abdominal hysterectomy, partial vaginectomy, and retropubic colpourethropexy.

Prophylactic antibiotics were used at the discretion of the senior author (H. P. D.). Seven patients received no prophylaxis, while eight patients received various antibiotics before and after the operation. Three of these eight patients had medical indications for prophylactic antibiotics (mitral valve prolapse) (Table II).

Operative technique. While under general anesthesia, the patient was placed in the lithotomy position for insertion of a Foley catheter. The vagina was packed with a vaginal roll soaked in Proviodine. The patient was then placed in the supine position for cleansing and draping of the abdomen. If a retropubic colpourethropexy was planned, the patient was placed in the semilithotomy position.

Concurrent procedures planned along with the vault suspension were performed first. Thus eight patients had retropubic colpourethropexies, four had internal anterior repairs (an equilateral triangle, approximately 5 cm on each side, was excised from the anterior vaginal wall through the abdomen, the redundant bladder base was plicated, and the vaginal walls were closed with a running locked 1-0 chromic suture), two had abdominal hysterectomies, and three had partial vaginectomies.

For the vaginal vault suspensions, the vaginal vault was freed up by dissecting the bladder anteriorly and the posterior peritoneum inferiorly. Three to four 1-0 Tevdek sutures (Deknatel, Queens Village, New York) were placed in the posterior aspect of the vagina, with care taken not to penetrate the mucosa. A piece of Marlex mesh, approximately 5 by 8 cm, was attached to the vagina with the aid of the sutures. Next the rectum and sigmoid colon were mobilized to the left, and the parietal peritoneum over the sacral promontory was incised longitudinally. Care was taken to expose the median sacral artery and vein and to identify the right ureter and iliac vessels, thereby avoiding operative injury. Three to five Tevdek sutures were placed through the anterior longitudinal ligament of the spine, and these were attached to the other end of the mesh. The mesh was trimmed and the peritoneum closed over the mesh (Figs. 1 and 2). The peritoneum over the paracolic gutters was also closed with 2-0 chromic suture.

In one patient, A. P., the vaginal vault was so long that it readily reached the sacral promontory. The presacral peritoneum was not opened, and the sutures were placed directly through the peritoneum into what was thought to be the ligamentous tissue covering the sacrum without using any mesh.

Results

All operations were performed by one of us (H. P. D.). Follow-up time is 3 months to 93 months (mean, 28 months). There was one surgical failure in the patient who had the vaginal vault suspension

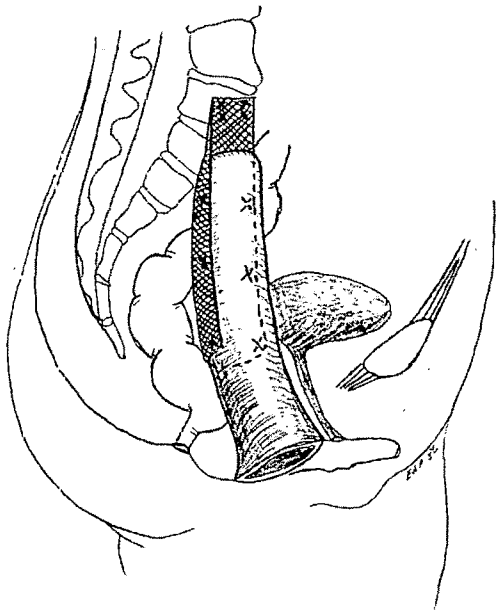


Fig. 1. Sagittal view showing mesh fixed to back of vagina and anchored to presacral fascia.

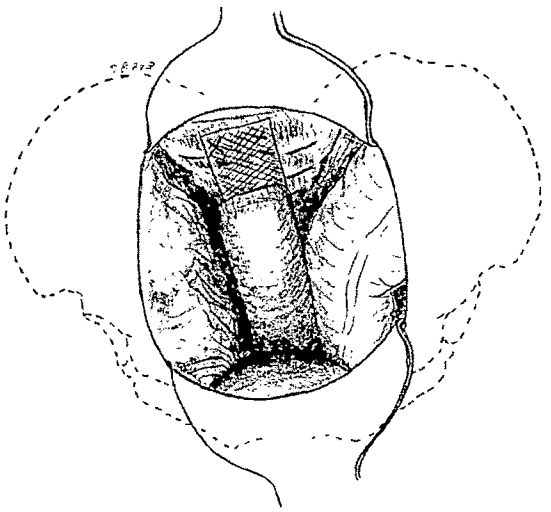


Fig. 2. View from above of vagina suspended to presacral fascia by Marlex mesh.

without mesh and without opening of the presacral peritoneum (A. P.). Vaginal vault prolapse recurred 6 months after operation. This patient was subsequently operated upon at another center, where an obliterative vaginal procedure was performed which corrected the defect but left her with a shortened nonfunctioning vagina.

One postoperative complication developed 3 weeks after operation in a patient who had a retroperitoneal colpourethropexy and vaginal vault suspension (W. E.). Fever and abdominal pain prompted a gallium scan, which showed bilateral retroperitoneal collections of pus. At operation, pus was evacuated from both iliopsoas muscles retroperitoneally, and cultures subsequently produced *Escherichia coli*. The Marlex mesh appeared free from the infectious process but was removed, since being a foreign body, it was thought to be a potential nidus of infection. The patient fully recovered, and the vaginal vault remained suspended.

Two patients developed small enteroceles and rectoceles. These did not cause any symptoms and have not been treated surgically.

Two patients with massive genital prolapse did very well postoperatively, and at follow-up 2 years after their operation, had well-supported vaginal vaults (Table III).

Use of prophylactic antibiotics appeared to decrease the incidence of febrile morbidity although not to a statistically significant degree. The infections in the retroperitoneal space and the abdominal wound occurred in patients who did not receive prophylactic antibiotics. At this point in time it is our clinical impression that prophylactic antibiotics should be used in this type of operation.

Comment

The present series of patients with vaginal vault prolapse represents an ongoing evaluation of the technique of abdominal suspension with use of Marlex mesh. The basic surgical technique has been described previously. Lane[2] reported four patients in whom Marlex mesh was used in vaginal vault suspension, but only the distal ends of the mesh were retroperitoneal, predisposing the patient to bowel obstruction. Soichet[3] described two patients in whom he buried the mesh completely, and Grundsell[4] reported 11 patients in whom Marlex mesh was placed retroperitoneally to suspend the vault. He attained excellent results (no recurrences, one cystocele).

Javey[5] described a completely extraperitoneal abdominovaginal operation for vaginal inversion, with use of Mersilene mesh in 13 patients without complications.

In our series the sacral promontory is used as the site of fixation because it is technically easier to place sutures through the anterior longitudinal ligament at this level. No problems with hemorrhage were encountered.

Suture placement at the hollow of the sacrum (S2-4)[6, 7] is complicated by the presence of presacral venous and nerve plexus, and life-threatening hemorrhage has been reported during this procedure.[8]

It has been shown by Nichols[9] that the vaginal axis assumes an almost horizontal plane in its upper two-thirds, where it lies on top of the rectum and levator plate. When attached to the sacral promontory, the vagina can still assume a normal axis because the mesh is not pulled taut.

Table IV. Review of literature

Authors	Year of publication	Procedure	No. of cases	Follow-up	Success		Significant complications
					n	%	
Lane	1962	Abdominal	20	Not reported	18/20	90	Not reported
Birnbaum	1973	Abdominal Vaginal	9 8	1-18 yr	9/9	100	7/8 = 87.5% nonfunctioning vagina; 1 incontinence
Ridley	1976	Abdominovaginal	12	>1 yr	12/12	100	Not reported
Nichols	1982	Vaginal	163	>2 yr	158/163	96.9	12/163 = 7.4%; 3 nonfunctioning vagina, 5 recurrent prolapse, 2 sciatic nerve trauma, 1 laceration of rectum, 1 death (pulmonary embolism)
Richter	1982	Vaginal	97 (83.5%)	>10 yr	95/97	97.9	2 recurrent prolapse, 29 enterocele, rectocele, incontinence
Grundsell	1984	Abdominal	9	Average, 3.9 yr	9/9	100	1 cystocele
Drutz, Cha	1987	Abdominal (Marlex)	15	3-93 mo; average, 28 mo	14/15	93.3	1 recurrent prolapse, 1 retroperitoneal abscess

Other techniques of posterior vaginal vault suspension have tried to restore the normal vaginal axis. Transvaginal fixation of the vault to the sacrospinous ligament[10, 11] (usually the right) restores the vaginal axis, but the technique can be fraught with difficulties. "Blind" placement of sutures can predispose the patient to injuries of pudendal vessels, sciatic nerve, urinary tract, and rectum. Also, the vagina may be shortened, impairing sexual function.[6] In the skilled hands of Nichols, however, this technique has given good results with few complications. Transvaginal fixation of the vault to the sacrotuberous ligament has been used infrequently because of its technical difficulty.

Table IV summarizes the results of these techniques of posterior suspension of the vaginal vault. Nichols[11] reported 12 complications in 163 patients including three patients with shortened vaginas, five with recurrent prolapses, two with sciatic nerve trauma, and one with laceration of the rectum. One patient died as a result of postoperative pulmonary embolism. In a series of 97 patients, Richter[10] reported two recurrent vault prolapses and 29 patients who subsequently developed enterocele, rectocele, or urinary incontinence. Birnbaum[6] had no recurrent case of vault prolapse, but one patient developed urinary incontinence. Grundsell[4] had complete success with his procedures, and only one patient of nine developed a cystocele. Lane[2] reported no complications in his short follow-up.

In summary, we feel that abdominal sacropexy is an excellent technique for the treatment of vaginal vault and massive genital prolapse. The advantage of an abdominal versus a vaginal approach is seen in less intraoperative injury to vessels, nerves, and bowel, since sutures can be placed under direct vision. Also, the abdominal approach allows for retention of vaginal length and strengthening of the pelvic floor and cul-de-sac. Coexisting defects of the vaginal wall can be corrected by internal anterior and/or posterior repair, and retropubic colpourethropexy can be performed for stress urinary incontinence at the same surgical setting. Marlex mesh has proved to be a strong and reliable synthetic bridge which we use frequently in suburethral slings and combined abdominovaginal slings for recurrent stress urinary incontinence; it has not previously been reported as a cause of infections.

REFERENCES

1. Ridley JH. A composite vaginal vault suspension using fascia lata. Am J Obstet Gynecol 1976;126:590.
2. Lane FE. Repair of post-hysterectomy vaginal vault prolapse. Obstet Gynecol 1962;2:72.
3. Soichet S. Surgical correction of total genital prolapse with retention of sexual function. Obstet Gynecol 1969;36:69.
4. Grundsell H. Operative management of vaginal vault prolapse following hysterectomy. Br J Obstet Gynecol 1984; 91:808.
5. Javey H. An extraperitoneal operation for vaginal vault suspension to the anterior abdominal wall. Br J Obstet Gynecol 1979;86:243.
6. Birnbaum S. Rational therapy for the prolapsed vagina. Am J Obstet Gynecol 1973;115:411.
7. Hendee AE. Abdominal sacropexy for vaginal vault prolapse. Clin Obstet Gynecol 1981;24:1217.
8. Sutton GP. Life-threatening hemorrhage complicating sacral colpopexy. Am J Obstet Gynecol 1981;140:836.
9. Nichols DH. Significance of restoration of normal vaginal depth and axis. Obstet Gynecol 1970;36:251.
10. Richter K. Massive eversion of the vagina. Clin Obstet Gynecol 1982;25:897.
11. Nichols DH. Sacrospinous fixation for massive eversion of the vagina. Am J Obstet Gynecol 1982;142:901.

Sacrococcygeal teratoma: Prenatal diagnosis and management

Susan J. Gross, M.D., Ronald J. Benzie, M.B., Ch.B., Mathew Sermer, M.D., Martin B. Skidmore, M.B., and Stephanie R. Wilson, M.D.

Toronto, Ontario, Canada

Although sacrococcygeal teratoma is a rare and potentially malignant tumor, 10 cases were documented during a 5-year period at the University of Toronto Perinatal Complex. Diagnosis was made in the six cases in which prenatal ultrasound examination was performed. One patient with twins elected to terminate the pregnancy at 19 weeks. In three of the cases diagnosed prenatally, serial ultrasound was performed. There was a 75% cesarean section rate. In all cases diagnosed prenatally, the large tumor size affected the mode of delivery. In the four cases without prenatal diagnosis, two infants were delivered vaginally, and two were delivered abdominally for obstetric reasons. There was one case of neonatal morbidity where tumor vascularity and rupture resulted in hypovolemic shock. All tumors were resected and found to be benign. A plan of management is recommended and, with appropriate obstetric and pediatric care, a good outcome can be anticipated in most cases. (AM J OBSTET GYNECOL 1987;156:393-6.)

Key words: Teratoma, sacrococcygeal, pregnancy

Sacrococcygeal teratoma is a congenital tumor arising from all three germ cell layers. It has an incidence of one to two per 40,000 deliveries[1] with only isolated reports in the literature. The importance of neonatal diagnosis is twofold. Ten percent of these tumors may be malignant at the time of delivery.[2] They may grow to a large size, affecting fetal outcome and labor management. The objectives of this study were to examine the prenatal diagnosis and the antepartum and intrapartum management with subsequent outcome.

Methods

Cases of sacrococcygeal teratoma during a 5-year period (from 1980 through 1985) from four tertiary referral centers forming the University of Toronto Perinatal Complex were studied retrospectively. Six cases, including one set of twins, originated from the complex itself, while four were referred from outlying hospitals. Prenatal assessment, diagnosis, fetal weight and sex, size of tumor, mode of delivery, and outcome were documented. A diagnosis of sacrococcygeal teratoma required histologic confirmation.

Results

A total of 10 cases were recorded, with one set of female twins and a female:male ratio of 9:1. In six of the cases, ultrasonographic diagnosis of sacrococcygeal teratoma was made, a large-for-dates uterus being the indication for the ultrasound examination. No other phenotypic abnormalities were identified although there were three cases of polyhydramnios. Because of prenatal diagnosis of sacrococcygeal teratoma in both twins, one patient requested and obtained termination of pregnancy at 19 weeks (Figs. 1 and 2). The remaining four cases were diagnosed at the time of delivery. The maternal serum α-fetoprotein and amniotic fluid α-fetoprotein and acetylcholinesterase had not been tested. There was a 50% (four of eight) prematurity rate. The overall average gestational age was 35.5 ± 4 weeks. There was a 75% (six of eight) cesarean section rate. In the four cases where prenatal diagnosis was made by ultrasound, the presence of a large tumor (average volume 2340 ± 1026 cm^3) affected the mode of delivery. In none of these cases was the infant delivered immediately after the diagnosis was made. In three of them, serial ultrasound examinations were performed and the fetus was delivered abdominally because of an abnormal nonstress test, premature rupture of the membranes, and polyhydramnios, respectively. In the four cases without prenatal diagnosis, two infants were delivered vaginally. The average volume of these tumors was relatively small (average volume 317 ± 197 cm^3). The remaining two infants were delivered by cesarean section for obstetric reasons (transverse lie and failure to progress, respectively, Table I).

Significant morbidity was seen in one case. Because of the large size and vascularity of the tumor, the infant developed hypovolemic shock during the resection operation resulting in subsequent pre-renal failure. Fig. 3 illustrates the size and vascularity of the teratoma. The infant received appropriate management and was normal at follow-up 2 years later.

From the University of Toronto Perinatal Complex (Toronto General Hospital, Women's College Hospital, Mount Sinai Hospital, and The Hospital for Sick Children).

Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.

Reprint requests: Dr. R. J. Benzie, Ottawa General Hospital, 501 Smyth, Ottawa, Ontario, Canada KIH 8L6.



Fig. 1. Long-axis ultrasound of twin A, showing caudal unilocular cystic mass.

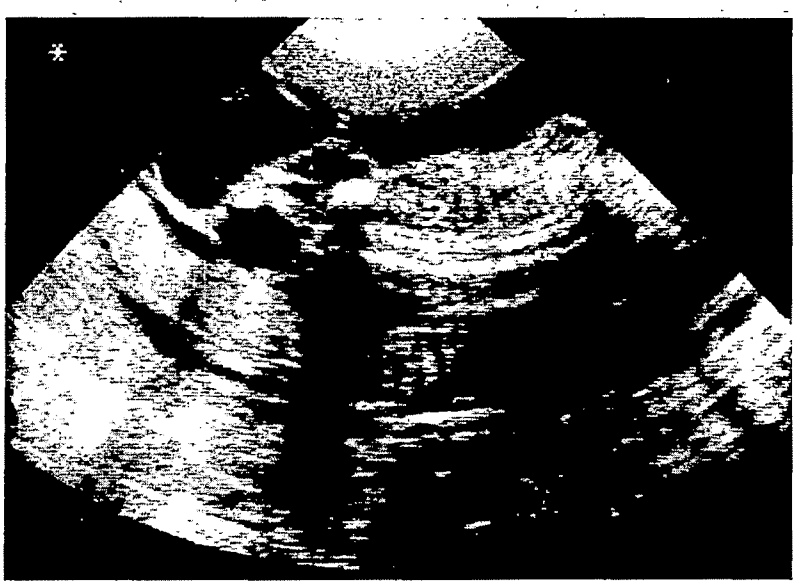


Fig. 2. Long-axis ultrasound of twin B, showing large caudal multilocular cystic mass.

There was one neonatal death secondary to prematurity and one death at 3½ years of age secondary to malignant endodermal sinus tumor, apparently unrelated to the original lesion.

The tumor was resected during the first week of life in surviving neonates and all the tumors were benign. No associated anomalies were found prenatally by ultrasound or at subsequent operation.

Comment

Our series was consistent with some known features of sacrococcygeal teratoma. It is more common in female infants (75% to 80%)[2] and there is concordance between twins.[3] As with many congenital malformations, sacrococcygeal teratoma is associated with polyhydramnios.[4] None of the tumors in our series, however, were malignant. No associated anomalies of the lower vertebrae, anorectum, or genitourinary system were documented.

With respect to prenatal diagnoses, while none of the cases were tested for elevated amniotic fluid α-fetoprotein and acetylcholinesterase levels, these enzymes have been found to be elevated in sacrococcygeal teratoma, especially in the second trimester.[5] Although elevation of these enzymes would not likely have affected the diagnosis in these cases, further study in this area is



Fig. 3. Female newborn infant with sacrococcygeal teratoma, 32 weeks' gestation, after cesarean section.

Table I. Summary of cases of sacrococcygeal teratoma

Case No.	*Fetal sex*	*Birth weight (gm)*	*Gestational age at diagnosis (wk)*	*Gestational age at delivery (wk)*	*Prenatal diagnosis*	*Tumor size at birth (cm)*	*Mode of delivery*
1	F	5000	23	36	Ultrasonographic	27 by 15 by 8	Cesarean section
2	F	2300	24	34	Ultrasonographic	14 by 10 by 10	Cesarean section
3	(Twin A) F		19	19	Ultrasonographic	1.5 by 1.5	Elective abortion
4	(Twin B) F		19	19	Ultrasonographic	6 (diameter)	Elective abortion
5	F	4600	30	32	Ultrasonographic	25 by 11 by 13	Cesarean section
6	F	2070	27	27	Ultrasonographic	5 by 5 by 5	Cesarean section
7	F	3960	40	40	—	10 by 7 by 7	Vaginal
8	M	5460	40	40	—	17 by 15 by 17	Cesarean section
9	F	2910	37	37	—	5 (diameter)	Cesarean section
10	F	3160	38	38	—	6 by 4 by 5	Vaginal

warranted. As seen in our series, where no ultrasonographic errors were made, careful ultrasonography can be a very accurate method of prenatal diagnosis and assessment. The important differential diagnosis of meningomyelocele and to a lesser extent hemangioma should not be forgotten.

We therefore recommend that, while a rare entity, sacrococcygeal teratoma should be suspected in a large-for-dates patient, and ultrasound examination should be performed in order to make the diagnosis prenatally. These patients should be evaluated by serial ultrasonography to assess tumor size. Appropriate counseling at the time of diagnosis is essential, not only in order to discuss possible termination but also to adequately educate and prepare parents for the impending labor, delivery, and management.

The literature documents different modes of management: (1) vaginal delivery,[3] (2) cesarean section,[4] (3) cesarean section after partial delivery because of dystocia,[6] (4) vaginal drainage of tumor followed by vaginal delivery,[7] and (5) abdominal drainage of tumor followed by vaginal delivery.[8] While mode of delivery has been individualized in the literature, tumor size is critical in this decision for two reasons. In our two cases of vaginal delivery, the tumors were small enough for delivery; however, a large tumor can cause serious obstetric complications such as abnormal lie and dystocia. More importantly, as seen in our one case of hypotensive shock, a large sacrococcygeal teratoma can be highly vascular, thereby causing exsanguination or rupture on aspiration. The literature documents cases of neonatal death after rupture of sacrococcygeal teratoma during vaginal delivery.[9]

On the basis of the above literature review and our own series of cases, we feel it reasonable to suggest that, except for very small tumors (≤5 cm in diameter), cesarean section affords the obstetrician maximal control.

Thus, with prior knowledge, adequate preparation can be made with respect to obstetric management. Appropriate medical and surgical pediatric care can

be obtained with good outcome anticipated in most cases.

We would like to thank Dr. Yung and Dr. Claessens for providing cases for this study.

REFERENCES

1. Salaymel MT. Giant sacrococcygeal teratoma in the newborn. Int Surg 1971;56:56.
2. Seeds JW, Mittelstaedt CA, Cefalo RC, Parker TF. Prenatal diagnosis of a sacrococcygeal teratoma—an anechoic caudal mass. JCU 1982;10:193.
3. Lees RF, Williamson BRJ, Brenbridge ANAG, Buschi AJ, Teja K. Sonography of benign sacral teratoma in utero. Radiology 1980;134:717.
4. Horger EO, McCarter LM. Prenatal diagnosis of sacrococcygeal teratoma. AM J OBSTET GYNECOL 1979;134:228.
5. Brock DJH, Richmond DH, Liston WA. Normal second-trimester amniotic fluid alphafetoprotein and acetylcholinesterase associated with fetal sacrococcygeal teratoma. Prenat Diagn 1983;3:343.
6. Musci MC, Clark MJ, Ayres RE, Finkel MA. Management of dystocia caused by a large sacrococcygeal teratoma. Obstet Gynecol 1982;62:105.
7. Weiss DB, Wajntraub G, Abulafia Y, Schiller M. Vaginal surgical intervention for a sacro-coccygeal teratoma obstructing labour. Acta Obstet Gynecol Scand 1976;55:183.
8. Tanaree P. Delivery obstructed by sacrococcygeal teratoma. AM J OBSTET GYNECOL 1982;142:239.
9. Guigiaro A, Boario U, Fraceso G, Freni G. Three cases of intrapartum rupture of sacrococcygeal teratoma [in Italian]. Minerva Pediatr 1977;29:1517.

Human chorionic gonadotropin, prolactin, estriol, and dehydroepiandrosterone sulfate concentrations in cord blood of premature and term newborn infants: Relationship to the sex of the neonate

Basil Ho Yuen, M.B., Ch.B., and Everett K. Mincey, Ph.D.
Vancouver, British Columbia, Canada

Although the composition of human chorionic gonadotropin, prolactin, estriol, and dehydroepiandrosterone sulfate in the umbilical cord blood has been studied, less information is available on the effect of fetal sex on the cord blood concentrations. We assayed these hormones in cord blood from 405 newborn infants delivered between 23 and 43 weeks of gestation. In pooled data for both sexes, human chorionic gonadotropin showed a declining trend from the beginning to the end of the sampling interval. By contrast, in 398 newborn infants of known sex, prolactin, estriol, and dehydroepiandrosterone sulfate levels increased during the same period. Sex differences were observed for human chorionic gonadotropin only; female newborn infants had higher cord blood concentrations throughout the sampling period, with the differences becoming statistically significant between 29 and 36 weeks. (AM J OBSTET GYNECOL 1987; 156:396-400.)

Key words: Newborn infants, sex, chorionic gonadotropin, prolactin, estriol, dehydroepiandrosterone sulfate, cord blood

Human chorionic gonadotropin, prolactin, estriol, and dehydroepiandrosterone sulfate are important polypeptide and steroid hormones produced in the fetal-placental unit. Although their composition in the umbilical cord blood has been studied, less information is available on the effect of fetal sex on the cord blood concentrations.[1, 2] In the present study, we measured the levels of these hormones in the cord blood of newborn infants from midpregnancy to term to determine whether there were any sex differences in these levels.

From the Gynaecologic Endocrine Laboratory, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynaecology, Faculty of Medicine, The University of British Columbia, and Metropolitan Biomedical Laboratories.
Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.
Reprint requests: Dr. B. Ho Yuen, Department of Obstetrics and Gynaecology, The University of British Columbia, 4490 Oak St., Vancouver, British Columbia, Canada V6H 3V5.

Material and methods

At the time of delivery, mixed umbilical cord blood was collected into tubes containing ethylenediamine-

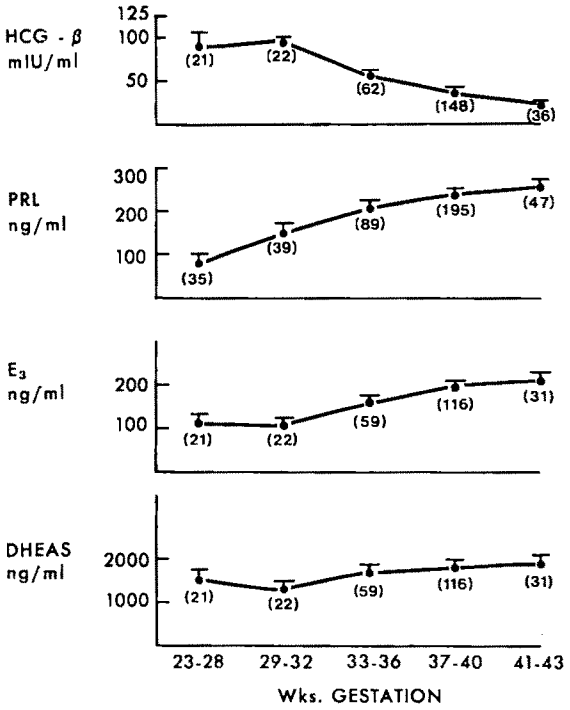


Fig. 1. Human chorionic gonadotropin *(HCG-β)*, prolactin *(PRL)*, estriol *(E_3)*, and dehydroepiandrosterone sulfate *(DHEAS)* concentrations in pooled umbilical cord blood of all newborn infants (n = 405). Number of samples assayed shown in parentheses.

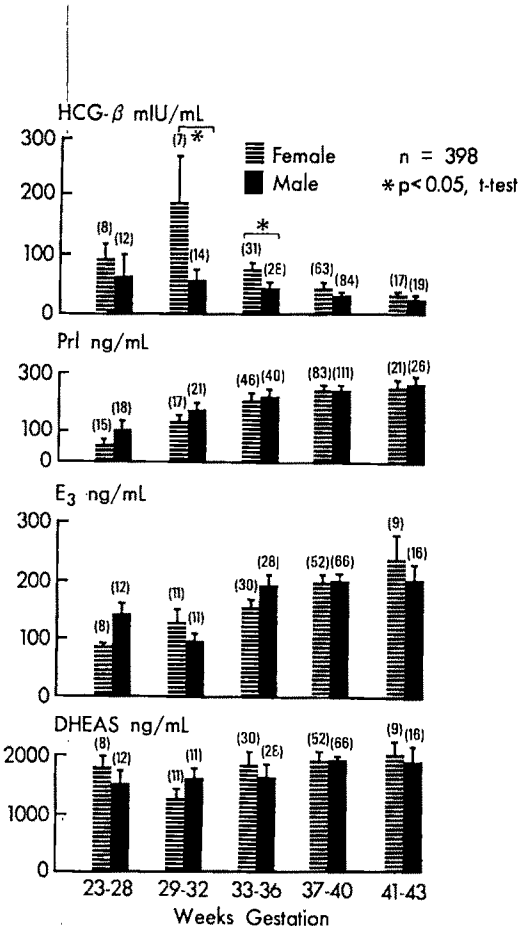


Fig. 2. Human chorionic gonadotropin *(HCG-β)*, prolactin *(PRL)*, estriol *(E_3)*, and dehydroepiandrosterone sulfate *(DHEAS)* concentrations in cord blood of newborn infants of known sex (n = 398) analyzed separately. Number of samples assayed shown in parentheses.

Table I. Duration of gestation, birth weight, human chorionic gonadotropin, prolactin, estriol, and dehydroepiandrosterone sulfate concentrations in cord blood of female and male newborn infants pooled

	Weeks of gestation				
	23-28	*29-32*	*33-36*	*37-40*	*41-43*
Weeks	26.4 ± 0.3 (35)	30.8 ± 0.2 (39)	34.7 ± 0.1 (89)	38.8 ± 0.1 (195)	41.4 ± 0.1 (47)
Birth weight (gm)	964.3 ± 43.8 (35)	1578 ± 47.3 (39)	2393.2 ± 48.5 (89)	3070.8 ± 38.1 (195)	3496.8 ± 65.5 (47)
Human chorionic gonadotropin (mIU/ml)	90 ± 28 (21)	95 ± 31 (22)	58 ± 7 (62)	36 ± 5 (148)	26 ± 4 (36)
Prolactin (ng/ml)	81 ± 19 (35)	157 ± 14 (39)	211 ± 13 (89)	245 ± 8 (195)	261 ± 13 (47)
Estriol (ng/ml)	119 ± 13 (21)	112 ± 12 (22)	165 ± 11 (59)	201 ± 9 (116)	208 ± 19 (31)
Dehydroepiandrosterone sulfate (ng/ml)	1575 ± 162 (21)	1384 ± 135 (22)	1747 ± 137 (59)	1883 ± 67 (116)	1961 ± 124 (31)

Data are shown as mean ± SEM; total number of newborn infants studied is shown in parentheses for weeks of gestation and birth weight; number of samples assayed for each hormone is also shown in parentheses.

tetraacetic acid as anticoagulant. Plasma was separated and stored frozen at −25° C until radioimmunoassay of the hormones.[3-5] The prolactin data from 405 newborn infants were reported in relationship to the presence or absence of respiratory distress syndrome together with that of estradiol and thyroid hormones.[6] The development of respiratory distress syndrome did not affect the results of prolactin determinations or the remaining hormone levels reported here. The data were accordingly pooled without regard to the presence

Table II. Human chorionic gonadotropin, prolactin, estriol, and dehydroepiandrosterone sulfate concentrations in cord blood of female and male newborn infants analyzed separately

	Weeks of gestation					
	23-28		*29-32*		*33-36*	
Sex	*Female*	*Male*	*Female*	*Male**	*Female*	*Male**
Human chorionic gonadotropin (mIU/ml)	93 ± 26 (8)	64 ± 38 (12)	188 ± 81 (7)	54 ± 19 (14)	74 ± 11 (31)	44 ± 7 (28)
Prolactin (ng/ml)	58 ± 14 (15)	105 ± 35 (18)	134 ± 19 (17)	175 ± 21 (21)	208 ± 19 (46)	222 ± 20 (40)
Estriol (ng/ml)	89 ± 14 (8)	141 ± 20 (12)	130 ± 22 (11)	100 ± 9 (11)	152 ± 14 (30)	190 ± 20 (28)
Dehydroepiandrosterone sulfate (ng/ml)	1764 ± 214 (8)	1504 ± 242 (12)	1216 ± 188 (11)	1600 ± 178 (11)	1838 ± 205 (30)	1629 ± 188 (28)

Data are shown as mean ± SEM; number of samples assayed for each hormone is shown in parentheses.

or absence of this disease. Medications of various types were used during pregnancy and labor involving 161 newborn infants. These were isoxsuprine in 68 (42%), glucocorticoids in 21 (13%), and various other agents, singly and in combination in the remaining neonates exposed to medications. In addition, numerous complications of pregnancy, including diabetes mellitus and hypertension, were also encountered, as previously described.[6] Data on the sex of seven newborn infants were not available, leaving a total of 398 infants from whom hormone data were obtained for analysis according to the sex of the infant. Data were analyzed by use of the *t* test.

Results

Data for all newborn infants are shown in Table I and Fig. 1. The data are shown separately for male and female infants in Table II and Fig. 2.

Cord blood levels of human chorionic gonadatropin were 90 ± 28 mIU/ml (mean ± SEM, second international standard) between 23 and 28 weeks of gestation and 95 ± 31 mIU/ml between 29 and 32 weeks; levels declined to 26 ± 4 mIU/ml toward the end of the sampling period at 41 to 43 weeks. Prolactin levels were 81 ± 19 and 261 ± 13 ng/ml; estriol, 119 ± 13 and 208 ± 19 ng/ml; and dehydroepiandrosterone sulfate, 1575 ± 162 and 1961 ± 124 ng/ml, respectively, at the beginning and end of the sampling interval. Sex differences were seen for human chorionic gonadotropin only, with female infants having higher cord blood values throughout the period studied between 23 and 43 weeks of gestation. Concentrations of human chorionic gonadotropin were significantly higher in female infants between 29 and 36 weeks of gestation.

Comment

The data presented indicate that umbilical cord blood concentrations of human chorionic gonadotropin show essentially a declining trend from midpregnancy to term. In contrast, prolactin, estriol, and dehydroepiandrosterone sulfate levels increased during the same interval. Sex differences were observed for human chorionic gonadotropin only. Female infants had significantly higher levels of human chorionic gonadotropin between 29 and 36 weeks of gestation.

For human chorionic gonadotropin, these data are in agreement with those of Boroditsky et al.,[7] Brody and Carlstrom,[8] and Danzer et al.,[9] indicating that circulating maternal levels of human chorionic gonadotropin were higher in women bearing female fetuses compared with women bearing male fetuses. Crosignani et al.[10] reported no sex differences in human chorionic gonadotropin levels of female and male infants between 38 and 41 weeks of gestation in their study. Our data showed higher levels of human chorionic gonadotropin for female infants over male infants throughout the period of 23 to 43 weeks of gestation; however, the data were statistically significant ($p < 0.05$) only between 29 and 36 weeks. In this context, our results are consistent with those of Crosignani et al.[10] inasmuch as neither study observed statistically significant differences in the interval from 37 weeks to term. The higher human chorionic gonadotropin levels in the cord and maternal blood in pregnancy with a female fetus are also associated with higher "female" amniotic fluid levels, compared with "male" amniotic fluid concentrations.[10] Furthermore, Wide and Hobson[11] also found that "female" placental tissue contains more human chorionic gonadotropin than "male" placental tissue. Because umbilical arterial progesterone concentrations are higher in male fetuses, Boroditsky et al.[7] suggested that progesterone may regulate production of human chorionic gonadotropin. In support of this interpretation, Wilson et al.[12] found that progesterone suppressed production of human chorionic gonadotropin from explants of human term placenta.

Weeks of gestation			
37-40		*41-43*	
Female	*Male*	*Female*	*Male*
43 ± 10	32 ± 4	30 ± 7	22 ± 5
(63)	(84)	(17)	(19)
244 ± 10	247 ± 9	254 ± 21	268 ± 17
(83)	(111)	(21)	(26)
197 ± 12	200 ± 11	238 ± 39	201 ± 26
(52)	(66)	(9)	(16)
1900 ± 109	1904 ± 86	2015 ± 188	1960 ± 174
(52)	(66)	(9)	(16)

In vivo and in vitro observations suggest that prolactin may also inhibit production of human chorionic gonadotropin from trophoblast near term.[13] The temporal relationship between the rising concentrations of prolactin and the declining levels of human chorionic gonadotropin in the fetal compartment (Table I and Fig. 1) is consistent with a possible inhibitory effect of prolactin noted. However, because sex differences for prolactin could not be demonstrated, it seems unlikely that prolactin is playing an important role in the increased human chorionic gonadotropin levels in female fetuses (Table II, Fig. 2).

Our results agree with those of Tapaininen et al.[14] and Badawi et al.[15] in which sex differences for prolactin could not be found. With respect to estriol and dehydroepiandrosterone sulfate, our results are also consistent with those of Laatikainen and Peltonen[16] who found that concentrations of these steroids were not influenced by the sex of the newborn infant.

Human chorionic gonadotropin may have adrenocorticotropic activity before 20 weeks of gestation. In the anencephalic fetus, the adrenal glands develop normally until 20 weeks; thereafter, the adrenal glands start to undergo atrophy in the presence of normal levels of human chorionic gonadotropin and prolactin.[2] The normal development of the adrenal glands of the anencephalic fetus before 20 weeks of gestation may be sustained by the high human chorionic gonadotropin levels produced by the trophoblast in early pregnancy.[2] Atrophy of the anencephalic fetal adrenal glands in later pregnancy in the presence of normal human chorionic gonadotropin and prolactin levels is consistent with adrenocorticotropic hormone being the major hormone after midpregnancy.[2] Fetal pituitary prolactin content is low before 15 weeks of gestational age but increases rapidly from this period to 23 weeks. Prolactin has also been proposed as a possible adrenocorticotropic hormone in the fetus.[2] The observation of normal prolactin levels in the anencephalic fetus with atrophic adrenal glands suggests that it is unlikely that prolactin has an important adrenocorticotropic role in utero.[2] The major secretory product of the fetal adrenal gland is dehydroepiandrosterone sulfate, which is the precursor of estriol in the fetal-placental unit. Glucocorticoids administered to the mother inhibit adrenocorticotropic hormone secretion with a decrease in the production of dehydroepiandrosterone sulfate and estriol. Although we do not know the effect of the various maternal complications of pregnancy or of the drugs used during pregnancy and labor on the hormone results reported here, our data appear to confirm and extend previous observations.

In conclusion, mean cord blood human chorionic gonadotropin concentrations from pooled data of both male and female newborn infants show a declining trend from 23 weeks of gestation to term. In contrast, mean cord blood levels of prolactin, estriol and dehydroepiandrosterone sulfate show an increasing trend during the same interval of pregnancy. Sex differences were observed for human chorionic gonadotropin only. The mean concentrations of human chorionic gonadotropin in the cord plasma of female newborn infants exceeded that of male newborn infants between 23 and 43 weeks of gestation; between 29 and 36 weeks of gestation, this difference was statistically significant.

REFERENCES

1. Lauritzen C, Klopper A. Estrogens and androgens. In: Fuchs F, Klopper A, eds. Endocrinology of pregnancy. Philadelphia: Harper & Row, 1983:73.
2. Gluckman PD, Grumbach MM, Kaplan SL. The human fetal hypothalamus and pituitary gland. In: Tulchinsky D, Ryan KJ, eds. Maternal-fetal endocrinology. Philadelphia: WB Saunders, 1980:196.
3. Ho Yuen B, McComb P, Sy L, Lewis J, Cannon W. Plasma prolactin, human chorionic gonadotropin, estradiol, testosterone, and progesterone in the ovarian hyperstimulation syndrome. Am J Obstet Gynecol 1979;133:316.
4. Ho Yuen B, Cannon W, Lewis J, Sy L, Woolley S. A possible role for prolactin in the control of human chorionic gonadotropin and estrogen secretion by the fetoplacental unit. Am J Obstet Gynecol 1980;136:286.
5. Ho Yuen B, Robertson J, Clement P, Mincey EK. Sclerosing tumor of the ovary. Obstet Gynecol 1982;60:252.
6. Ho Yuen B, Powell Phillips WD, Cannon W, Sy L, Redford D, Burch P. Prolactin, estradiol, and thyroid hormones in umbilical cord blood of neonates with and without hyaline membrane disease: a study of 405 neonates from midpregnancy to term. Am J Obstet Gynecol 1982;142:698.
7. Boroditsky RS, Reyes FI, Winter JSD, Faiman C. Serum chorionic gonadotropin and progesterone patterns in the last trimester of pregnancy: relationship to fetal sex. Am J Obstet Gynecol 1975;121:238.
8. Brody S, Carlstrom G. Human chorionic gonadotropin pattern in serum and its relation to the sex of the fetus. J Clin Endocrinol Metab 1965;25:792.
9. Danzer H, Braunstein GD, Rasor J, Forsythe A, Wade ME. Maternal serum human chorionic gonadotropin concentrations and fetal sex prediction. Fertil Steril 1980;34:336.
10. Crosignani PG, Nencioni T, Brambati B. Concentrations of chorionic gonadotrophin and chorionic somatomammotrophin in maternal serum, amniotic fluid, and cord

blood serum at term. J Obstet Gynaecol Br Commonw 1972;79:122.
11. Wide L, Hobson B. Relationship between the sex of the fetus and the amount of human chorionic gonadotrophin in placentae from the 10th to the 20th week of pregnancy. J Endocrinol 1974;61:75.
12. Wilson EA, Jawad MJ, Dickson LR. Suppression of human chorionic gonadotropin by progestational steroids. AM J OBSTET GYNECOL 1980;138:708.
13. Ho Yuen B, Moon YS, Shin DH. Inhibition of human chorionic gonadotropin production by prolactin from term human trophoblast. AM J OBSTET GYNECOL 1986; 154:336.
14. Tapaininen J, Huhtaniemi I, Koivisto M, Kujansuu E, Tuimala R, Vihko R. Hormonal changes during the perinatal period: FSH, prolactin and some steroid hormones in the cord blood and peripheral serum of preterm and full-term female infants. J Steroid Biochem 1984;20: 1153.
15. Badawi M, Van Exter C, Delogne-Desnoeck J, Van Meenen F, Robyn C. Cord serum prolactin in relation to the time of day, the sex of the neonate and the birth weight. Acta Endocrinol 1978;87:241.
16. Laatikainen T, Peltonen J. Levels of estriol, estriol sulfate, progesterone, and neutral steroid mono- and disulfates in umbilical cord arterial and venous plasma. In: Forest MG, Bertrand J, eds. International symposium on sexual endocrinology of the perinatal period. Paris: INSERM, 1974:255.

Differing concentrations of human chorionic gonadotropin and prolactin in the cyst fluid of hydatidiform mole and in amniotic fluid

Basil Ho Yuen, M.B., Ch.B.
Vancouver, British Columbia, Canada

The concentrations of human chorionic gonadotropin and prolactin in the cyst fluid of molar tissue were compared with that of normal amniotic fluid. Molar fluid was aspirated from the vesicles of molar tissue in eight women (duration of amenorrhea 14.1 ± 1 week, mean ± SEM). Amniotic fluid was obtained at amniocentesis in 24 women (mean duration of amenorrhea 15.9 ± 0.2 week). Hormones were measured by specific radioimmunoassay. The concentrations (mean ± SEM) of human chorionic gonadotropin in molar fluid and amniotic fluid were 581,829 ± 112,581 and 3187 ± 505 mIU/ml ($p < 0.001$), respectively. For prolactin the levels in molar fluid and amniotic fluid were 44 ± 24 and 1962 ± 313 ng/ml ($p < 0.001$), respectively. These data demonstrate that molar fluid contains 182-fold higher levels of human chorionic gonadotropin and 45-fold lower levels of prolactin than amniotic fluid obtained from normal pregnant women with a similar duration of amenorrhea. In addition to altered endocrine function of the trophoblast, there may be altered prolactin secretion from the decidua in molar pregnancy as compared with normal pregnancy. Further research is required to evaluate prolactin production from decidua of molar pregnancy. (AM J OBSTET GYNECOL 1987;156:400-2.)

Key words: Human chorionic gonadotropin, prolactin, molar cyst fluid

During normal pregnancy human chorionic gonadotropin levels in amniotic fluid are lower than those in the maternal blood.[1] Prolactin levels in amniotic fluid rise as gestation progresses to peak in the midtrimester and then decline toward term.[1,2] The source of human chorionic gonadotropin in normal and molar pregnancy is the trophoblast.[3] The source of prolactin in the amniotic fluid has not been clearly defined. Recent studies[4,5] demonstrate that the decidua secretes prolactin and that the mechanisms controlling its secretion from this tissue differs from that of the pituitary gland.[6] It has been suggested that the decidua is an important source of amniotic fluid prolactin.[4,5] The physiologic role of prolactin in amniotic fluid has not been defined. It has been suggested, however, that prolactin may have an osmoregulatory role in the formation of amniotic fluid.[1,4] The concentrations of prolactin in molar vesicular fluid have not previously been determined.

From the Gynaecologic Endocrine Laboratory, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynaecology, Faculty of Medicine, University of British Columbia, and Grace Hospital.
Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.
Reprint requests: Basil Ho Yuen, M.B., Ch.B., 4490 Oak St., Vancouver, British Columbia, Canada V6H 3V5.

Table I. β-Subunit of human chorionic gonadotropin and prolactin concentrations in amniotic fluid and molar cyst fluid

	Amniotic fluid (n = 24 women)	*Molar cyst fluid (n = 8 women)*	*p*
β-Subunit of human chorionic gonadotropin* (mIU/ml)	3,187 ± 505	580,829 ± 102,581	<0.001
Prolactin (ng/ml)	1,962 ± 313	44 ± 24	<0.001

Data are shown as mean ± SEM.
*Second international standard.

The purpose of the present study was to determine whether the concentrations of these peptide hormones in the amniotic fluid of normal pregnancy differed from those of molar pregnancy at approximately comparable intervals of gestation.

Material and methods

Amniotic fluid was obtained at the time of amniocentesis from 24 women for indications based on maternal age. The women were 36.2 ± 0.5 years of age (mean ± SEM). The duration of amenorrhea at the time of amniocentesis was 15.9 ± 0.2 weeks. Only patients whose amniotic fluid karyotype findings were normal were included in the study. In addition, amniotic fluid samples contaminated with blood or meconium were discarded. Molar cyst fluid was aspirated from the vesicles in the operating room immediately after surgical evacuation of the uterus with the patient under general anesthesia. Only samples that were free of contamination by blood were used. The mean age of the eight women with molar pregnancy was 25 ± 2.5 years and the duration of amenorrhea at the time of evacuation was 14.6 ± 1 week. The amniotic fluid and molar cyst fluid specimens were centrifuged before being stored frozen at −20° C before the hormones were measured by radioimmunoassay. Assay kits for the assay of the β-subunit of human chorionic gonadotropin were purchased from Amersham Canada Limited, Oakville, Ontario, Canada, and prolactin assay kits were purchased from Becton Dickinson Immunodiagnostics, Mississauga, Ontario, Canada. The between-assay coefficient of variation for assay of the β-subunit of human chorionic gonadotropin was 10% while the within-assay coefficient of variation was 8%. For the prolactin assay the between- and within-assay coefficients of variation were 11% and 5%, respectively. Data were analyzed by the Mann-Whitney test.

Results

The duration of amenorrhea in women with normal pregnancy at the time of amniocentesis was not significantly different from that of the women with molar pregnancy at the time of uterine evacuation. However, the women undergoing amniocentesis were significantly older ($p < 0.05$) than the women with evacuation of a molar pregnancy.

Hormone results are shown in Table I.

Comment

The human chorionic gonadotropin and prolactin concentrations of amniotic fluid reported are in agreement with those of other authors.[1-3] The mean human chorionic gonadotropin level in amniotic fluid was 3187 mIU/ml while in molar cyst fluid the mean human chorionic gonadotropin level was 580,829 mIU/ml. The mean prolactin levels in amniotic fluid and molar cyst fluid were 1962 and 44 ng/ml, respectively.

In the present study the human chorionic gonadotropin levels in the molar cyst fluid were about 180-fold higher than those of normal amniotic fluid for the same stage of amenorrhea. In contrast, the prolactin levels in molar cyst fluid were 45-fold lower than those of amniotic fluid for similar stages of amenorrhea. These findings suggest that, in addition to the altered endocrine function of the trophoblast, there is altered prolactin secretion within molar pregnancy as compared with that in the fetal-placental compartment of normal pregnancy. Unlike amniotic fluid, the molar cyst fluid is characterized by high human chorionic gonadotropin and low prolactin concentrations. Since trophoblast is the major source of human chorionic gonadotropin secretion, the higher human chorionic gonadotropin levels in molar cyst fluid reflect the excess production of human chorionic gonadotropin by molar trophoblast. As compared with normal placental tissue of comparable duration of pregnancy, hydatidiform mole tissue has been shown to have a higher human chorionic gonadotropin content.[3] Although the reasons for the lower prolactin levels in molar pregnancy cyst fluid are not known, they may include altered production from the decidua. Should this be so, the decidua of molar pregnancy may share a feature with decidua of spontaneously aborting embryopathic pregnancy, where the production of prolactin appears to be somewhat less than that of normal pregnancy.[7] As previously noted, in normal pregnancy, the decidua may

be an important source of amniotic fluid prolactin.[4, 5] Further research is required to determine if prolactin production from the decidua of molar pregnancy is altered. Other possible explanations for the observations presented may include mechanisms representing an altered net clearance of prolactin and human chorionic gonadotropin from the trophoblast-decidua complex of molar pregnancy as compared with that of the normal fetal-placental unit. Furthermore, the role of the fetus in the distribution of these hormones in the normal fetal-placental unit has not been elucidated.

In conclusion, the vesicular fluid of molar pregnancy, as compared with that of normal amniotic fluid, contains a great deal more human chorionic gonadotropin but much less prolactin. In addition to altered endocrine function of trophoblast in molar pregnancy,[3] prolactin secretion from the decidua may also be altered. Further research is required to evaluate prolactin production from decidua of molar pregnancy.

I thank Dr. L. Kirby for samples of amniotic fluid.

REFERENCES

1. Belisle S, Tulchinsky D. Amniotic fluid hormones. In: Tulchinsky D, Ryan KJ, eds. Maternal-fetal endocrinology. Philadelphia: WB Saunders, 1980.
2. Gluckman PD, Grumbach MM, Kaplan SL. The human fetal hypothalamus and pituitary gland. In: Tulchinsky D, Ryan KJ, eds. Maternal-fetal endocrinology. Philadelphia: WB Saunders, 1980.
3. Van Leusden HA. Chorionic gonadotropin in pathological pregnancy. In: Klopper A, ed. Plasma hormone assays in evaluation of fetal well being. New York: Churchill Livingstone, 1976.
4. Riddick DH, Luciano AA, Kusmik WF, Maslar IA. De novo synthesis of prolactin by human decidua. Life Sci 1978;23:1913.
5. Golander A, Hurley T, Barrett J, Hizi A, Handwerger S. Prolactin synthesis by human chorion-decidua tissue: a possible source of prolactin in the amniotic fluid. Science 1978;202:311.
6. Golander A, Barrett J, Hurley T, Barry S, Handwerger S. Failure of bromocriptine, dopamine, and thyrotropin-releasing hormone to affect prolactin secretion by human decidual tissue in vitro. J Clin Endocrinol Metab 1979; 49:787.
7. Kuboto T, Suzuki A, Yaoi Y, Kumasaka T, Saito M. Study on plasma prolactin and immunoreactive-prolactin released from decidua in normal pregnancy and abortion. Asia Oceania J Obstet Gynaecol 1984;10:255.

Doppler ultrasound prediction of fetal outcome in twin pregnancies

C. Nimrod, M.B., D. Davies, R.D.M.S., J. Harder, M.D., C. Dempster, R.D.M.S., G. Dodd, R.N., N. McDicken, Ph.D., and S. Nicholson, M.D.
Calgary, Alberta, Canada

The usefulness of pulsed Doppler ultrasound in predicting twin pregnancies destined for unsatisfactory outcomes was examined in 30 twin pregnancies. Fetal flow velocity waveform analysis (systolic/diastolic ratio and pulsatility index) and quantitative analysis in the descending aorta and umbilical artery demonstrated good sensitivity and specificity when compared with biparietal diameter and abdominal circumference measurements. This technique shows some promise as a useful adjunct in the management of twin pregnancies. (Am J Obstet Gynecol 1987;156:402-6.)

Key words: Twin pregnancies, outcome, pulsed Doppler ultrasound

Twin gestation is associated with a significant increase in perinatal morbidity and mortality. This increase is mainly attributed to premature delivery and growth discrepancy.[1] Ultrasound is a useful tool in the assessment of growth problems; however, the sensitivity of biparietal diameter differences of >7 mm in the same twin gestation in predicting growth failure has been reported at only 20%.[2] This limitation has emphasized the need for a better diagnostic test to identify pregnancies at risk for compromise. The usefulness of flow velocity waveform analysis in the assessment of fetal health has been addressed previously.[3] Increased vascular impedance downstream from the point of Dopp-

From the Departments of Obstetrics, Radiology, and Pediatrics, University of Calgary Medical School and Foothills Hospital.
This work was supported by the Max Bell Foundation.
Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.
Reprint requests: Dr. C. Nimrod, Division of Maternal-Fetal Medicine, Foothills Hospital, University of Calgary, 1403-29th St. N.W., Calgary, Alberta, Canada T2N 2T9.

Table I. Characteristics of patients with unsatisfactory outcomes

Patient No.	*Gestational age at delivery (wk)*	*Birth weight difference (gm)*	*Below tenth percentile*	*Biparietal diameter difference >7 mm*
1	26	15	+	−
2	34	700	+	+
3	37	760	+	−
4	36	820	+	−
5	38	570	+	−
6	37	800	+	+
7	38	460	+	+
8	26	160	−	+
9	37	675	+	−
10	32	40	−	−
11	28	110	+	−

ler sampling will cause decreased diastolic flow, which will result in changes in both the peak systolic/end diastolic ratio and the $\frac{\text{peak systolic–end diastolic}}{\text{mean flow}}$ ratio, that is, the pulsatility index.[4] This study prospectively evaluated the changes occurring in blood flow in twin gestations and assessed the value of these measurements in predicting pregnancies destined to produce unsatisfactory outcomes.

Material and methods

Consecutive patients who presented to the ultrasound department with twin pregnancies beyond 20 weeks' gestation were recruited into this study. Fetal Doppler ultrasound research was approved for study in our institution. Routine biometric parameters including biparietal diameter and abdominal circumference were measured according to the methods reported by Hadlock et al.[5,6] Pulsed Doppler evaluation of the descending aorta and umbilical artery of each twin was conducted during periods of absent fetal breathing with the use of a Toshiba SAL 50A (Toshiba America, Tustin, California) with a 2.42 MHz pulsed Doppler transducer attached to a 3.5 MHz linear array transducer at a fixed angle of 55 degrees. A 100 Hz thump filter was used. The system was calibrated by the method of McDicken[7] to ensure that the quantitative estimate of flow was accurate. Patients were either in a supine position or at a slight left lateral tilt during these studies. The fetal descending thoracic aorta was examined just above the level of the diaphragm with the aorta oriented parallel to the transducer. A large enough sample volume was chosen to completely cross the vessel but not provide interference from other vessels. The aortic diameter and Doppler angle were then measured and entered into the machine to allow online calculation of mean velocity and volume flow. The umbilical artery was examined with the position of the sample volume adjusted to provide the best signal. No corrections were made for the Doppler angle and vessel diameter. Freeze-frame images of aortic and umbilical flow velocity waveforms were then used for off-line analysis on a microcomputer with a digitizing pad (Microsonics, Indianapolis, Indiana). Peak systolic and end diastolic velocities were marked by hand for measurement as these end points were clearly visible. Three consecutive waveforms were measured and a simple average calculated. The percent differences between the twin pairs was calculated for each parameter except biparietal diameter, for which millimeter differences were calculated in order to allow for comparison with previous reports.[2]

Pregnancy outcome was correlated with the results of the last ultrasound examination before delivery. Any of the following was considered an unsatisfactory outcome: (1) preterm delivery before 32 weeks' gestation, (2) major birth weight discrepancy of >500 gm, (3) birth weight below the tenth percentile for gestational age,[8] or (4) admission to the neonatal intensive care unit for problems originating in the antenatal period. The Doppler ultrasound information was blinded to the clinicians managing the patients.

Results

Thirty patients were recruited into the study and 11 of these were classified as having an adverse outcome (Table I) (<32 weeks' gestation at delivery, two; below tenth percentile, nine; weight discrepancy >500 gm, six; admission to neonatal intensive care unit, seven). The mean gestational age at the time of the examination was 31.7 weeks for the normal group and 31.8 weeks for the abnormal pairs with the mean gestation at delivery being 37.0 and 34.5 weeks, respectively, for the normal and abnormal groups. There was a statistically significant difference in the mean birth weights between the normal (2560 ± 357 gm) and the abnormal (1810 ± 796 gm) twin pairs ($p < 0.002$). Five infants from the abnormal group died in the perinatal period, while there were no losses in the group classified as normal. Also no significant congenital abnormality was identified in the entire study population. The in vitro calibration of the Doppler system demonstrated

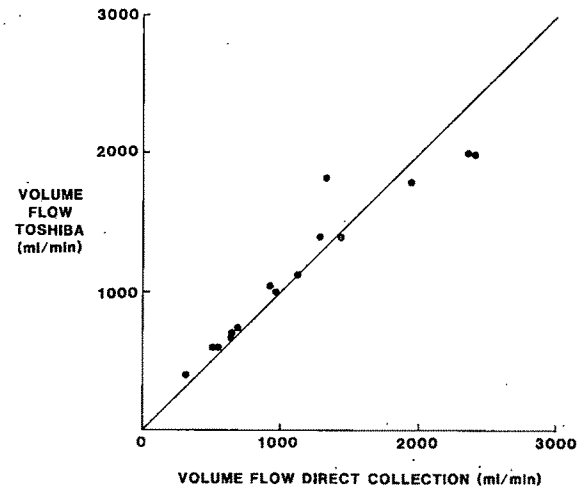


Fig. 1. In vitro calibration of Toshiba Doppler system at different volume flows.

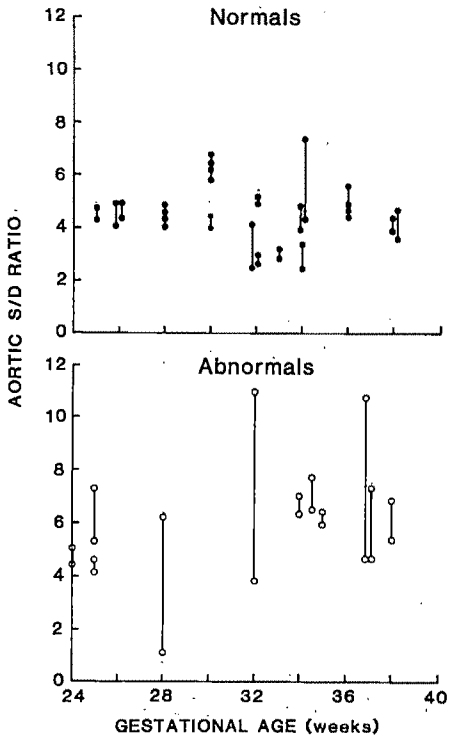


Fig. 2. Aortic peak systolic/end diastolic *(S/D)* ratio at different gestational ages in normal and abnormal twin pairs.

a systematic error of 5% in quantitative flow over a wide range of velocities (Fig. 1).

In two patients, both abdominal circumference and Doppler study of the fetal aorta could not be obtained. The umbilical peak systolic/end diastolic ratio was unobtainable in five different patients classified in the abnormal group and in six patients with normal outcomes. This was due in large part to the difficulty in

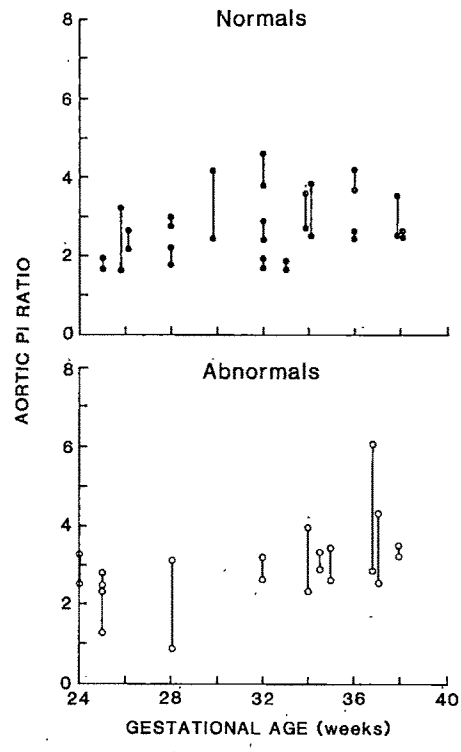


Fig. 3. Aortic pulsatility index *(PI)* at different gestational ages in normal and abnormal twin pairs.

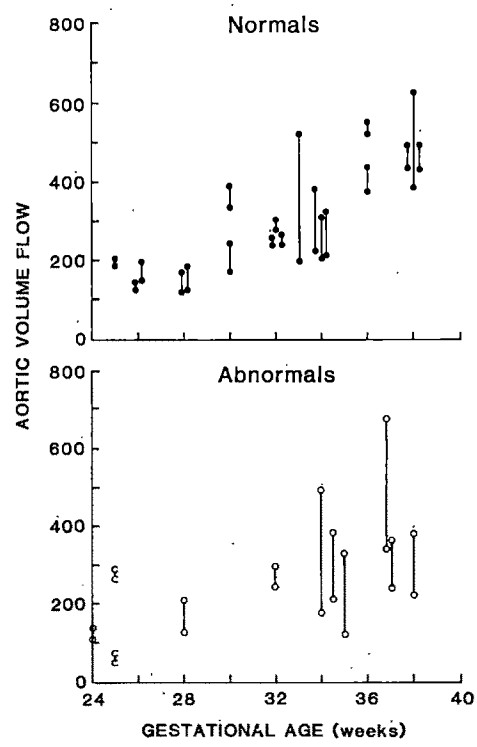


Fig. 4. Aortic volume flow at different gestational ages in normal and abnormal twin pairs.

Table II. Differences for each parameter in normal and abnormal twin pairs

		Difference (%)							
			Doppler parameter						
	Difference in biparietal diameter (mm)	*Abdominal circumference*	*Peak systolic velocity*	*Peak diastolic velocity*	*Mean velocity*	*Volume flow*	*Aortic peak systolic/end diastolic ratio*	*Aortic pulsatility index*	*Umbilical peak systolic/end diastolic ratio*
Normal group									
Mean	3.4	5.3	20.0	21.4	24.4	34.6	16.8	26.2	50.3
1 SD	2.9	3.6	20.2	22.0	30.2	36.0	18.9	27.5	82.6
Abnormal group									
Mean	7.5	7.0	23.6	106.8	37.2	84.8	92.6	65.1	55.4
1 SD	4.7	4.8	23.3	112.6	41.7	50.4	119.5	77.1	34.4
Sensitivity	45	22	22	67	22	56	44	44	50
Specificity	84	74	89	84	89	89	89	84	82

identifying which fetus belonged to the cord sampled.

The normal and abnormal twin pair data for aortic peak systolic/end diastolic (Fig. 2), aortic pulsatility index (Fig. 3), and aortic volume flow (Fig. 4) are plotted against gestational age to highlight the differences seen in the individual twin pairs. When abnormal twin pairs are examined, aortic peak systolic/end diastolic ratios accentuate the differences to a greater extent than the pulsatility index. Significant differences are also demonstrated in aortic blood flow volume in the abnormal group after 32 weeks' gestation. The mean percent difference for each parameter in normal and abnormal twin pairs is shown in Table II in conjunction with the standard deviations. In addition, the sensitivity and specificity for a biparietal diameter difference of ≥7 mm and abdominal circumference and Doppler parameters with differences of 1 SD above the mean are shown.

Comment

The umbilical artery has been the vessel most commonly assessed by Doppler ultrasound. This, however, does present a problem in twin gestation as it is not always easy to clarify which fetus is associated with the umbilical vessel under study. Studies on the descending aorta have been shown to be useful;[9] however, the volume flow calculation has had limited use because of the size of the error involved in the assessment of the cross-sectional area of a vessel.[10] The observation that volume flow differences in abnormal fetuses were exaggerated beyond 32 weeks' gestation is understandable in light of the dependence of volume flow on vessel diameter as a reflection of fetal size[11] and the acceleration of fetal growth that occurs around this gestational age.

The only report of Doppler evaluation of twin gestation used umbilical peak systolic/end diastolic ratios.[12] In this study, we attempted to expand this to include waveform analysis and volume flow in the fetal aorta and used a pulsed Doppler system rather than continuous wave. In addition, a new outcome category to include adverse fetal outcome without significant weight discrepancy was used.

This study compares the twins in the form of percent difference. This approach has some limitations as abnormal situations can be masked if both twins are similarly compromised and hence, would have relatively similar blood flow changes. This did not occur in any of our cases but underlines the need to compare the absolute Doppler measurements to the normal range in all cases. For abdominal circumference four of nine patients in the abnormal group had a value below the tenth percentile for our laboratory, but in only two was a significant difference between pairs noted.

In our study, a biparietal diameter difference of >7 mm was equivalent to 1 SD from the mean, and although the sensitivity of 45% is better than the sensitivity of 20% reported by Leveno et al.,[2] it is still too low to be useful as a screening test for predicting unsatisfactory outcome. It does, however, have a low false positive rate of 16%. Abdominal circumference difference was not of value although absolute values were somewhat better. When considered individually, the Doppler parameters all have similar specificities but the sensitivity of the mean velocity and systolic peak velocity was too low to be useful. End diastolic flow and volume flow appear more sensitive than the remaining parameters but are still too low to be useful screening tests on their own. Pulsatility index generated values similar to the peak systolic/end diastolic ratio and was probably testing a similar physiologic parameter. It is unlikely that it would provide more information about the state of the fetal circulation.

Combining parameters has been used effectively for detecting intrauterine growth retardation and forms the basis of well-accepted indices such as the biophysical profile and Apgar score. When biparietal diameter, peak end diastolic velocity, volume flow, and aortic and

umbilical peak systolic/end diastolic are considered together, nine of 11 in the abnormal group had two or more of these parameters outside the normal range, and in the other two cases one or more of the Doppler parameters could not be obtained. This combination of parameters improves the sensitivity to 82%. The specificity, however, is lowered as the false positive rate goes up to 30%. Measurement of several parameters also helps to overcome the technical difficulties encountered in obtaining good Doppler studies in both twins. In all cases, either the umbilical artery or the aorta could be satisfactorily assessed.

In conclusion, Doppler evaluation of fetal vessels is simple and noninvasive and can be performed by skilled sonographers usually in less than 30 minutes. It shows promise as a useful discriminator of birth weight differences and poor outcome in twin pregnancies. Use of several parameters appears to increase the sensitivity even though quantitative analysis (volume flow) may be difficult. Flow velocity waveform analysis should therefore be considered in the management of twin pregnancies.

REFERENCES

1. Cetrulo CL, Ingardia C, Sbana A. Management of multiple gestation. Clin Obstet Gynecol 1980;23:533-48.
2. Leveno KJ, Santos-Ramos R, Duenhoelter J. Sonar cephalometry in twin pregnancy: discordancy of the biparietal diameter after 28 weeks' gestation. Am J Obstet Gynecol 1980;138:615-9.
3. Trudinger BJ, Giles WB, Cook CM. Uteroplacental blood flow velocity-time waveforms in normal and complicated pregnancy. Br J Obstet Gynaecol 1985;92:39-45.
4. Trudinger BJ, Giles WB, Cook CM, et al. Fetal umbilical artery flow velocity waveforms and placental resistance: clinical significance. Br J Obstet Gynaecol 1985;92:23-30.
5. Hadlock FP, Deter RL, Harrist RB, et al. Fetal biparietal diameter: rational choice of plane of section for sonographic measurement. Am J Radiol 1982;138:871-4.
6. Hadlock FP, Deter RL, Harrist RB, et al. Fetal abdominal circumference as a predictor of menstrual age. Am J Radiol 1982;139:367-70.
7. McDicken N. A veratile test object for the calibration of ultrasonic Doppler instruments. Ultrasound Med Biol 1986;12:245-9.
8. Vital statistics: births and deaths. Ottawa, Ontario, Canada: Statistics Canada, 1980, vol 1:18-23.
9. Jouppila P, Kirkinen P. Increased vascular resistance in the descending aorta of the human fetus in hypoxia. Br J Obstet Gynaecol 1984;91:853-6.
10. Griffin D, Cohen-Overbeek T, Campbell S. Fetal and uteroplacental blood flow. Clin Obstet Gynaecol 1983;10:565-601.
11. Erskine RL, Ritchie JWK. Quantitative measurement of fetal blood flow using Doppler ultrasound. Br J Obstet Gynaecol 1985;92:600-24.
12. Giles W, Trudinger B, Cook C. Fetal umbilical artery flow velocity-time waveforms in twin pregnancies. Br J Obstet Gynaecol 1985;92:490-7.

Congenital and nosocomial sepsis in infants born in a regional perinatal unit: Cause, outcome, and white blood cell response

Arne Ohlsson, M.D., and Mary Vearncombe, M.D.
Toronto, Ontario, Canada

The incidence, cause, and outcome of sepsis and the white blood cell response were studied in 6315 infants born in a regional perinatal unit. The incidence of neonatal sepsis was 6.5 per 1000 live births. Congenital sepsis (12 cases) was overwhelming, with associated maternal infection (92%), neutropenia (75%), and high rate of mortality (50%). The most common organism was *Escherichia coli* (58%). Gestational age and birth weight were similar in survivors and nonsurvivors. There was a strong correlation between total white blood cell count and both mature and immature neutrophil counts in survivors but this correlation decreased substantially in neonates that died. Analysis of variance indicated that the means for polymorphonuclear leukocyte and immature neutrophil counts were significantly higher in survivors. Nosocomial sepsis (38 cases) occurred in premature low birth weight infants receiving invasive, intensive care. The most common organism was *Staphylococcus epidermidis* (76%). Total white blood cell, polymorphonuclear leukocyte, and immature neutrophil counts rose significantly in response to sepsis. None died. Prevention of congenital sepsis requires methods to detect early maternal-fetal infection. Providing granulocytes to neutropenic neonates with congenital sepsis might improve outcome. (AM J OBSTET GYNECOL 1987;156:407-13.)

Key words: Sepsis, congenital, nosocomial, neonate, infant, neutropenia, etiology, outcome

Sepsis is a generalized symptomatic infection confirmed by a positive blood culture. The incidence of sepsis occurring in the neonatal period (<28 days of age) varies from one to 10 per 1000 live births, and the mortality varies from 20% to 75%.[1,2] The handicap rate among survivors exceeds 25%.[2] The susceptibility to and outcome of sepsis relate to the type and number of invading organisms, the gestational age and weight of the neonate, the age at the onset of sepsis, the presence of malformations requiring operation, predisposing maternal conditions, and early diagnosis and therapy.[3,4] If neutropenia develops in a neonate with bacterial sepsis and a bone marrow aspirate demonstrates depletion of the neutrophil storage pool, the likelihood of survival is <20%.[5] Extensive reviews of the literature between 1927 and 1978 have shown that the cause of neonatal sepsis varies with geographic area and with time.[3,6] It is therefore important to frequently review the cause of neonatal sepsis locally.[3,4] The most commonly isolated organisms are *Escherichia coli*, other gram-negative organisms such as *Klebsiella, Enterobacter,* and *Serratia marcescens,* group B streptococci, and *Staphylococcus aureus.*[3,6] Recently an increasing incidence of nosocomial sepsis caused by *Staphylococcus epidermidis*[7-9] and *Candida albicans*[10,11] has been noted in neonatal intensive care units.

The purpose of this study was to determine the incidence, cause, and outcome of sepsis occurring among infants recently born in a regional perinatal unit. The white blood cell response to sepsis in these infants was also studied to establish the number of infants that might benefit from granulocyte transfusion.

Material and methods

Population studied. All infants with both clinical signs of sepsis and a positive blood culture, born in the University of Toronto Regional Perinatal Unit at Women's College Hospital, Toronto, Ontario, Canada, during 1984 and 1985, were studied.

Culture procedures. Blood cultures were obtained either from the umbilical artery at the time of the initial catherization at birth or from a peripheral vein after thorough preparation of the skin with iodine solution. Before September 1984, blood cultures were inoculated into a radiometric hypertonic aerobic medium (Bactec 8A, Johnston Laboratories) and incubated at 35° C for 7 days. From September 1984 to the end of the study period, blood for cultures was inoculated into pediatric Isolator tubes (DuPont) and plated onto chocolate agar and Brucella agar plates according to the manufacturer's instructions. Plates were incubated in 5% carbon dioxide and anaerobically, respectively, at 35° C for 4

From the University of Toronto Regional Perinatal Unit and the Department of Clinical Laboratories, Women's College Hospital.
Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.
Reprint requests: Arne Ohlsson, Regional Perinatal Unit, Women's College Hospital, 76 Grenville St., Toronto, Ontario, Canada M5S 1B2.

Table I. Clinical and laboratory findings in 12 episodes of congenital sepsis

Case No.	*Sex*	*Gestational age (wk)*	*Premature rupture of membranes*	*Maternal infection*	*Maternal hospital stay*	*Birth weight (gm)*	*Organism isolated*
1	M	32	—	Chorioamnionitis	16 hr	1710	*E. coli*
2	F	39	22 hr	Chorioamnionitis	20 hr	3720	*E. coli*
3	F	39	4 hr	Chorioanmionitis	6 hr	2935	*E. coli*
4	M	26	10 days	Chorioamnionitis	10 days	830	*E. coli*
5	M	28	9 days	Chorioamnionitis	10 days	1080	*E. coli*
6	M	28	3 days	Chorioamnionitis	2.5 days	1330	*E. coli*
7	M	33	9 wk	Chorioanmionitis	2 hr	2100	*E. coli*
8	F	27	5 days	Chorioamnionitis	2.5 days	870	*S. pneumoniae*
9	F	42	18 hr	Sepsis	1.5 days	3385	*Enterococcus*
10	F	25	5 days	Chorioamnionitis	4 days	630	Group B streptococcus
11	F	26	—	Elevated temperature	1.5 hr	760	*H. influenzae* type b
12	M	31	—	—	9 days	1580	*C. albicans*

I/T ratio, Immature/total neutrophil ratio.

Table II. Clinical and laboratory findings in 38 episodes of nosocomial sepsis

Case No.	*Sex*	*Gestational age (wk)*	*Birth weight (gm)*	*Age at diagnosis (days)*	*Organism grown*	*I/T ratio*	*Assisted ventilation*	*Hyperalimentation*	*Other site of positive culture*
1	F	26	660	14	*S. epidermidis*	0.21	+	+	—
2	F	27	900	11	*S. epidermidis*	0.41	+	+	—
3	F	27	660	26	*S. epidermidis*	0.08	+	+	—
4	F	25	670	12	*S. epidermidis*	0.18	+	+	Catheter
5	F	27	1010	7	*S. epidermidis*	0.14	+	+	—
6	F	28	960	12	*S. epidermidis*	0.62	+	+	Catheter
7	F	27	900	14	*S. epidermidis*	0.32	+	+	Endotracheal tube
8	F	34	1120	38	*S. epidermidis*	0.00	+	+	—
9	F	26	920	29	*S. epidermidis*	0.42			Endotracheal tube
10	F	25	590	14	*S. epidermidis*	0.28	+	+	Catheter
11	F	30	1260	17	*S. epidermidis* and *E. coli*	0.03	−	+	—
12	F	25	730	47	*E. coli*	0.05	+	+	—
13	F	24	750	15	*E. cloacae*	0.49	+	+	—
14	F	25	990	18	*E. cloacae*	0.27	+	+	—
15	M	30	1250	8	*S. epidermidis*	0.48	+	+	—
16	M	30*	1660	14	*S. epidermidis*	0.52	+	+	Radial arterial line
17	M	25	670	26	*S. epidermidis*	0.01	+	+	—
18	M	26	840	11	*S. epidermidis*	0.14	+	+	—
19	M	29	1090	10	*S. epidermidis*	0.15	+	+	Catheter
20	M	33	1530	9	*S. epidermidis*	0.19	−	+	—
21	M	27	1000	9	*S. epidermidis*	0.19	+	+	—
22	M	28	1120	19	*S. epidermidis*	0.07	+	+	—
23	M	32	1300	15	*S. epidermidis*	0.00	−	+	—
24	M	28	1300	18	*S. epidermidis*	0.06	+	+	—
25	M	31	1080	8	*S. epidermidis*	0.16	+	+	Catheter
26	M	32	1280	8	*S. epidermidis*	0.03	−	+	—
27	M	30	1530	14	*S. epidermidis*	0.12	+	+	—
28	M	26	820	9	*S. epidermidis*	0.12	+	+	—
29	M	26	900	23	*S. epidermidis*	0.24	+	+	—
30	M	26	820	34	*S. epidermidis*	0.18	+	+	—
31	M	26	850	47	*S. epidermidis*	0.16	+	+	Catheter
32	M	30*	1660	32	*S. epidermidis*	0.39	+	−	Catheter
33	M	26	890	45	*S. epidermidis*, 2 strains	0.27	−	−	—
34	M	28	1200	12	*S. epidermidis* and *Torulopsis glabrata*	0.15	−	+	Catheter, *S. epidermidis*
35	M	28	1080	17	*E. coli*	0.08	+	+	—
36	M	29	1480	54	*S. epidermidis*	0.41	−	−	Urine
37	M	27	1150	9	*S. epidermidis* and enterococcus	0.00	−	+	Endotracheal tube (*E. coli*)
38	M	28	970	79	*E. cloacae*	0.08	+	+	—

I/T ratio, Immature/total neutrophil ratio.

*Cases 16 and 32 are the same patient with two episodes of sepsis.

Neutropenia	*I/T ratio*	*Status*
+	0.58	Died at 5 days
−	0.56	Alive
+	0.91	Died at 12 hr
+	0.10	Died at 13 hr
+	0.41	Alive
+	0.29	Died at 3 days
+	0.89	Alive
+	0.12	Alive
−	0.11	Alive
−	0.74	Alive
+	0.25	Died at 3 days
+	0.60	Died at 10 days

days. Growth was quantitated as the number of colonies per milliliter of blood. In all cases of suspected sepsis cerebrospinal fluid was obtained for cell count, Gram stain, and culture. Appropriate cultures and Gram stains were performed on material from any focal infection. Central indwelling venous catheters were not used during the study period, but when nosocomial infection was suspected any peripheral vascular line was removed and cultured by a semiquantitative technique.[12]

Maternal risk factors. The following maternal risk factors known to be associated with neonatal sepsis were noted: infection, time between rupture of the membranes and delivery, and antenatal treatment with antibiotics and/or steroids. Maternal hospital stay before delivery was recorded in congenital sepsis. During the study period, unless contraindicated, all mothers in premature labor at 25 to 32 weeks' gestation were given Vasodilan (Mead Johnson & Co.) in an attempt to stop labor to allow time for the action of bethamethasone. They were given two 12 mg doses of betamethasone 12 hours apart to promote fetal lung maturation. If labor recurred after the bethamethasone effect had been established no attempt was made to stop labor in the presence of premature rupture of the membranes.

Mothers presenting with premature rupture of the membranes had an introital swab taken for culture for group B streptococci. They were then given one million units of penicillin G every 6 hours intravenously until results of cultures were available. The treatment was stopped if the cultures were negative and continued if positive until two consecutive cultures were negative. If an introital swab was positive for group B streptococci at any time during the pregnancy, treatment with penicillin was given during labor. Erythromycin was used instead of penicillin in case of penicillin allergy.

Blood work and criteria. Peripheral total white blood cell counts, polymorphonuclear leukocyte counts, and immature neutrophil counts were obtained in all infants at the time of suspected sepsis. In nosocomial sepsis these results were compared to results of tests that had been performed within the two preceding days. Granulocytopenia was defined according to the criteria established by Xanthou[13] as <1300 polymorphonuclear leukocytes per cubic millimeter. The immature/total neutrophil ratio was calculated according to Manroe et al.[14] Bone marrow aspiration to evaluate the neutrophil storage pool was not performed.

Treatment. All infants were given full life support as indicated. Ampicillin and gentamicin in combination were used initially in the treatment of suspected sepsis and the antibiotics were subsequently changed according to sensitivity of the bacterial isolates.

Statistical analysis. Statistical analysis comparing groups was performed with the use of a paired two-tailed Student's *t* test on the leukocyte response to nosocomial sepsis. In congenital sepsis a two-factor analysis of variance with repeated measures was used on the polymorphonuclear and immature neutrophil counts at birth and 24 hours after birth. Some correlation matrices were obtained on the above sets of data. These statistical procedures were carried out after a square root transformation.

Results

During the 2-year study period there were 6315 live births, and septicemia was diagnosed on 50 occasions (54 bacterial isolates) in 49 patients at age 0 to 79 days. Neonatal sepsis occurred on 41 occasions, an incidence of 6.5 per 1000 live births. Sepsis occurred on nine occasions in infants older than 28 days of age. Concomitant meningitis occurred in one infant with *E. coli* sepsis. None of the patients had a major congenital malformation, and none underwent operation. The overall mortality was 12%. Two distinct groups of septic infants could be identified.

Congenital sepsis. In group 1 (12 neonates) sepsis was congenital (Table I). The male/female ratio was 1/1. The mortality was 50% and neutropenia was common (75%). The immature/total neutrophil ratio was increased (>0.16) in 75% of neonates with congenital sepsis. All neonates that died were neutropenic and had an immature/total neutrophil ratio that varied between 0.1 and 0.9. Eleven of the twelve (92%) mothers who gave birth to a neonate with congenital infection also showed signs of infection (Table I). Eight had premature rupture of the membranes >12 hours (67%); eight (67%) were treated with steroids and six (50%) with penicillin before delivery. Eight mothers (67%) had been in the hospital >12 hours and seven (58%) >24 hours before delivery. There was no significant difference in gestation age or birth weight between survivors and nonsurvivors with congenital sepsis. *E. coli* was the most common organism isolated (58%). All bacteria causing congenital sepsis were sensitive to at least one of the routinely used antibiotics (ampicillin and gentamicin). The mortality rate was 50%.

Table III. White blood cell counts (mean ± SD) in survivors and nonsurvivors of congenital sepsis at birth and at 24 hours of age

	Nonsurvivors	*Survivors*
Day of birth		
No.	6	6
Total white blood cell count (No./mm³)	7,583 ± 6,585	18,300 ± 13,843
Polymorphonuclear leukocytes (No./mm³)	630 ± 454	6,587 ± 12,014
Immature neutrophils (No./mm³)	1,427 ± 2,464	4,301 ± 4,249
Second day of life		
No.	4	6
Total white blood cell count (No./mm³)	2,900 ± 1,042	19,650 ± 8,996
Polymorphonuclear leukocytes (No./mm³)	413 ± 577	8,496 ± 4,467
Immature neutrophils (No./mm³)	805 ± 693	5,190 ± 9,425

Nosocomial sepsis. In group 2 sepsis was nosocomial (37 infants with 38 septic episodes) with onset between 7 and 79 days (Table II). The clinical and laboratory findings were similar in late-onset neonatal sepsis and in sepsis occurring in infants older than 28 days. There were no episodes of sepsis diagnosed between 1 and 6 days of age. Therefore, all infants with sepsis presenting after birth were grouped and analyzed together as nosocomial sepsis. The male/female ratio was 1.7/1. There was no significant difference in mean gestational age between male (28.4 ± 2.2 weeks) and female (27 ± 2.6 weeks) infants, but male infants had a significantly higher mean birth weight (1145 ± 282 gm) compared with that of female infants (866 ± 196 gm) ($p < 0.005$).

There were no deaths in infants with nosocomial sepsis and neutropenia was always absent at the time of diagnosis. Sixty percent of the infants had an elevated immature/total neutrophil ratio (>0.12). Seventy-six percent of nosocomial bacterial isolates were *S. epidermidis* followed by *E. coli* (12%) and *Enterobacter cloacae* (7%). Sixty-three percent (20 of 32) of *S. epidermidis* isolates were resistant to methicillin. Bacterial isolates other than *S. epidermidis* were sensitive to either ampicillin or gentamicin. Twenty-four of the 37 mothers (65%) had been treated with steroids before delivery and 11 (30%) with antibiotics. Nine mothers (24%) had premature rupture of the membranes for >12 hours. There were no deaths.

White blood cell response. The white blood cell responses to congenital sepsis and nosocomial sepsis are presented in Tables III and IV. Three neonates with congenital sepsis (25%) and seven with nosocomial sepsis (18%) had a platelet count <150,000/mm³ at the time of diagnosis. Correlation matrices between total white blood cell counts, polymorphonuclear leukocyte counts, and immature neutrophil counts in survivors and nonsurvivors of congenital sepsis are presented in Table V and the same correlation matrices for nosocomial sepsis 2 days before diagnosis and on the day of diagnosis are presented in Table VI. The cell means for the model used in analysis of variance for white blood cell count at birth and 24 hours after birth in congenital sepsis are shown in Table VIIA, with the corresponding analysis of variance shown in Table VIIB.

Comment

The incidence of sepsis in this study corresponds to those of earlier recent reports.[1-4, 6] Multiple risk factors were present in mothers of neonates with congenital sepsis: maternal infection, premature rupture of the membranes, and antenatal treatment with steroids and antibiotics. Nine mothers had been in the hospital for more than 12 hours and seven for more than 24 hours but the maternal-fetal infection was either not suspected or suspected only at parturition in most cases. Antenatal maternal treatment with steroids may have contributed to the seriousness of the infection in the neonate. Presently the methods to detect maternal infection are crude and the most sensitive test, C-reactive protein, has a high sensitivity but a low specificity.[15] Innovative approaches to this maternal population could possibly modify the very high mortality and survivor morbidity of congenital sepsis. In our population congenital sepsis was caused either by virulent bacteria with capsular polysaccharide antigens or in one patient by *Candida albicans*. *E. coli* was the major pathogen, as shown in other reviews of the cause of neonatal sepsis.[3, 6] Group B streptococci were of minor importance in this study compared with its role described in the literature.[3, 6] This might be explained by the routine use of penicillin in mothers with premature rupture of the membranes, which could have selected other penicillin-resistant organisms. *Streptococcus pneumoniae, Haemophilus influenzae* type b, and *C. albicans*, each isolated once, rarely cause congenital sepsis.[3, 4] All bacteria causing congenital sepsis were sensitive to at least one of the two antibiotics that are routinely used in the initial treatment of suspected sepsis in our unit. Ampicillin and gentamicin in combination should therefore remain the antibiotics of choice in the treatment of congenital sepsis.

The importance of *S. epidermidis* as a cause of nosocomial sepsis was shown in this study. Gram-negative nosocomial sepsis was rare in our unit and our results differ considerably from those of a study by Whyte et al.[8] from the neonatal unit at the Hospital for Sick Children, Toronto. They studied nosocomial infections

Table IV. White blood cell count response (mean ± SD) in nosocomial sepsis

	Within 2 days before diagnosis	*On day of diagnosis*	*Significance*
Total white blood cell count (No./mm³)	15,588 ± 7,157	27,163 ± 13,738	$p < 0.0000004$
Polymorphonuclear leukocytes (No./mm³)	8,199 ± 5,674	15,702 ± 9,427	$p < 0.000006$
Immature neutrophils (No./mm³)	675 ± 907	4,449 ± 5,315	$p < 0.0000002$

Table V. Correlation matrices between total white blood cell count and polymorphonuclear and immature neutrophil counts in survivors and nonsurvivors

	Total white blood cell count	*Polymorphonuclear leukocyte count*	*Immature neutrophil count*
Matrix 1. Survivors			
Total white blood cell count	1.0000	—	—
Polymorphonuclear leukocyte count	0.8461	1.0000	—
Immature neutrophil count	0.7371	0.2859	1.0000
Matrix 2. Nonsurvivors			
Total white blood cell count	1.0000	—	—
Polymorphonuclear leukocyte count	0.5181	1.0000	—
Immature neutrophil count	0.8456	0.3595	1.0000

Table VI. Correlation matrices between total white blood cell count and polymorphonuclear and immature neutrophil count in nosocomial sepsis 2 days before diagnosis and on day of diagnosis

	Total white blood cell count	*Polymorphonuclear leukocyte count*	*Immature neutrophil count*
Matrix 1. 2 Days before diagnosis			
Total white blood cell count	1.000	—	—
Polymorphonuclear leukocyte count	0.6321	1.000	—
Immature neutrophil count	0.0282	−0.3220	1.0000
Matrix 2. Day of diagnosis			
Total white blood cell count	1.000	—	—
Polymorphonuclear leukocyte count	0.8495	1.0000	—
Immature neutrophil count	0.6311	0.3250	1.0000

in infants born at <32 weeks' gestation and weighing <1250 gm, who were admitted from the same catchment area as the mothers in our unit. In their study the most commonly isolated bacteria in nosocomial sepsis (33 infants) were *S. epidermidis* (24%), *Klebsiella pneumoniae* (24%), and *E. coli* (21%). Although the catchment area is the same, the populations differ in that at Women's College Hospital all infants are delivered in the unit, whereas the neonatal unit at the Hospital for Sick Children treats only referred patients, including a large number of patients with disorders requiring operation. Continuing surveillance of the bacterial flora in each neonatal unit is therefore important to ensure appropriate therapy.

S. epidermidis sepsis occurs in infants receiving intensive, invasive care including respiratory support and hyperalimentation. An intravenous infusion site is, in our experience, as well as in the experience of others,[9] the usual source of entry (Table II).

Two of our infants, who had no obviously infected infusion site, had growth of the same strain of *S. epidermidis* in their endotracheal tubes. The trachea could possibly be another site of entry when an endotracheal tube requiring frequent manipulation is present. A large percentage (63%) of *S. epidermidis* isolates were resistant to methicillin but all were sensitive to vancomycin. It is important for both diagnosis and therapy that any indwelling infusion line is removed as soon as sepsis is suspected. Treatment with vancomycin should be initiated when significant *S. epidermidis* bacteremia is a likely cause of sepsis in the clinical situation.[9]

The overall mortality was low (12%) compared with that of other studies.[1,2] The mortality in congenital sepsis was 50%, which compares with 42%,[4] 69%,[8] and 70%[7] in three recent studies. In contrast there was no mortality in nosocomial sepsis, which has a reported mortality of 12%,[7] 20%,[4] and 39%.[8]

It is well known that congenital sepsis is often associated with neutropenia, and this association carries a poor prognosis.[4,5,7] This was confirmed in our study, as death occurred only in neutropenic infants.

Christensen et al.[5] found that neonates with an immature/total neutrophil ratio in excess of 0.8 are likely to have critical diminution of marrow neutrophil re-

Table VIIA. Polymorphonuclear leukocyte and immature neutrophil count means, after square root transformation, at birth, and 24 hours later

State	*At birth*		*24 hr later*	
	Polymorphonuclear leukocyte count	*Immature neutrophil count*	*Polymorphonuclear leukocyte count*	*Immature neutrophil count*
Dead	21.39	19.11	16.98	20.04
Alive	61.32	56.44	89.27	52.08

Table VIIB. Analysis of variance

Source of variation	*Degrees of freedom*	*Mean square*	*F*	*Tail probability*
State (dead/alive)	1	19781.59678	12.26	0.0081
Time (birth/24 hr)	1	242.43708	0.23	0.6444
Cell type*	1	1023.40869	0.42	0.5342
Time · cell type	1	436.17562	1.01	0.3453

*Polymorphonuclear leukocyte count/immature neutrophil count.

serves, which can be confirmed by a marrow aspirate. In our study only four neonates (two died and two survived) had an immature/total neutrophil ratio >0.8 at birth or at 24 hours of age. In congenital sepsis there was a high correlation between total white blood cell counts and both polymorphonuclear leukocyte counts and immature neutrophil counts in survivors, but this correlation decreased substantially in neonates that died (Table V), indicating that these neonates were not able to mount a neutrophil cell response to sepsis. As seen in Table VIIB the main effect of state (dead-alive) was the only significant result ($p < 0.0081$). Thus analysis of variance confirmed that the groups that survived and died of congenital sepsis differed in regard to polymorphonuclear leukocyte counts and immature neutrophil counts. The means for polymorphonuclear leukocyte counts and immature neutrophil counts across the living group were substantially higher than the means in the group that died. Short of marrow aspiration, an excellent predictor of outcome in congenital sepsis in our population was neutropenia at birth that persisted at 24 hours of age. Three of six survivors with congenital sepsis were neutropenic at birth but had a normal mature neutrophil count at 24 hours of age. In contrast all six infants that died were neutropenic at birth and by 24 hours they either had died (two) or were still neutropenic and would eventually die (four).

Infants with nosocomial sepsis were not neutropenic at the time of diagnosis but showed a highly significant rise in total white blood cell counts, polymorphonuclear leukocyte counts, and immature neutrophil counts compared with counts within 2 days before diagnosis (Table IV). In Table VI it is worth noting that the correlation between polymorphonuclear leukocyte counts and immature neutrophil counts changed direction between the two matrices, from −0.3220 in matrix 1 to 0.3250 in matrix 2. Also there was a substantial increase in the correlation between immature neutrophil counts and total white blood cell counts; the correlation increased from 0.0282 to 0.6311. In the neonates with nosocomial infection in this study, a marked increase in total white blood cell counts, polymorphonuclear leukocyte counts, and immature neutrophil counts thus occurred. In contrast this response was lacking in neonates that died of congenital infection.

This review shows that the causes of congenital sepsis differ between populations. In nosocomial sepsis the cause also differed from that found in another unit in the same city, even though the mothers-infants were recruited from the same catchment area. The present treatment of congenital sepsis in our unit with ampicillin and gentamicin is adequate in view of bacterial causes. As death occurred only in congenital sepsis, in spite of appropriate antibiotics, efforts to improve outcome should concentrate on prevention and innovative therapies. Methods to improve early detection of maternal-fetal infection are essential. This study identified a small number of neutropenic neonates with sepsis who may benefit from a treatment that would increase the number of mature granulocytes, by either exchange transfusion with fresh blood or granulocyte transfusion.[16] As these neonates are critically ill at birth and deteriorate rapidly, it is essential that all diagnostic tests and treatment modalities are readily available on a 24-hour basis in spite of the rare occurrence of neonates that would qualify for the treatment. It is also important that neonates subjected to any new type of treatment have long-term follow-up for evaluation of the ultimate effect of the treatment on perinatal morbidity and biologic-psychological-social handicaps.

REFERENCES

1. Siegel JD, McCracken GH. Sepsis neonatorum. N Engl J Med 1981;304:642-7.
2. Eriksson M. Neonatal septicemia. Acta Paediatr Scand 1983;72:1-8.
3. Ohlsson A, Serenius F. Neonatal septicemia in Riyadh, Saudi Arabia. Acta Paediatr Scand 1981;70:825-9.
4. Ohlsson A, Bailey T, Takieddine F. Changing etiology and outcome of neonatal septicemia in Riyadh, Saudi Arabia. Acta Paediatr Scand 1986;75:540-4.
5. Christensen RD, Bradley PP, Rothstein G. The leukocyte left shift in clinical and experimental neonatal sepsis. J Pediatr 1981;98:101-5.
6. Spiegelblatt L, Saintonge J, Chicoine R, Laverdiere M. Changing pattern of neonatal streptococcal septicemia. Pediatr Infect Dis 1985;4:56-8.
7. Placzek MM, Whitelaw A. Early and late neonatal septicemia. Arch Dis Child 1983;58:728-31.
8. Whyte H, Read S, Szymonowicz W, Pape K. Neonatal nosocomial infection [Abstract 304]. In: Clinch J, Matthews T, eds. Abstracts of the ninth European congress of perinatal medicine. Dublin, Ireland: September 2-5, 1984.
9. Noel GJ, Edelson PJ. *Staphylococcus epidermidis* bacteremia in neonates: further observations and the occurrence of focal infection. Pediatrics 1984;74:832-7.
10. Johnson DE, Thompson TR, Green TP, Ferrieri P. Systemic candidiasis in very low-birth-weight infants (<1,500 grams). Pediatrics 1984;73:138-43.
11. Baley JE, Kliegman RM, Fanaroff AA. Disseminated fungal infections in very low-birth-weight infants: clinical manifestations and epidemiology. Pediatrics 1984;73:144-52.
12. Maki DG, Weise CE, Sarafin HW. A semiquantitative culture method for identifying intravenous-catheter-related infection. N Engl J Med 1977;296:1305-9.
13. Xanthou M. Leukocyte blood picture in healthy full-term and premature babies during neonatal period. Arch Dis Child 1970;45:242-9.
14. Manroe BL, Weinberg AG, Rosenfeld CR, Browne R. The neonatal blood count in health and disease. I. Reference values for neutrophilic cells. J Pediatr 1979;95:89-98.
15. Ismail MA, Zinaman MJ, Lowensohn RL, Moawad AH. The significance of C-reactive protein levels in women with premature rupture of membranes. AM J OBSTET GYNECOL 1985;151:541-4.
16. Christensen RD, Rothstein G, Anstall HB, Bybee B. Granulocyte transfusions in neonates with bacterial infection, neutropenia, and depletion of mature marrow neutrophils. Pediatrics 1982;70:1-6.

A complete list of references will be supplied on request to the authors.

Postmenopausal estrogen and androgen replacement and lipoprotein lipid concentrations

Barbara B. Sherwin, Ph.D., Morrie M. Gelfand, M.D., Reuben Schucher, Ph.D., and Joseph Gabor, M.Sc.

Montreal, Quebec, Canada

The effect of a combined estrogen-androgen drug on the lipoprotein lipid profile of women who received it parenterally as long-term postmenopausal replacement therapy was compared to that of women who had been receiving estrogen alone parenterally and to untreated surgically menopausal women. Plasma estradiol, testosterone, total cholesterol, triglycerides, high-density lipoproteins, and low-density lipoproteins were measured at baseline (55 days after injection) and then on days 2, 4, 8, 15, 21, and 28 after injection. The plasma estradiol levels were greater in the two hormone-treated groups compared with the control group ($p < 0.01$), and plasma testosterone levels of the combined group exceeded those of the other two groups ($p < 0.01$) and remained above the normal female range for the duration of the study. There were no between-group differences in total cholesterol, triglycerides, high-density lipoprotein, or low-density lipoprotein values. Neither was the low-density/high-density lipoprotein ratio significantly different between groups at any of the test times. Therefore, the addition of testosterone to a long-term parenteral estrogen replacement regimen did not induce an increased atherogenic lipid profile compared with that produced by the parenteral administration of estrogen alone. (AM J OBSTET GYNECOL 1987;156:414-9.)

Key words: Estrogen, androgen, menopause, lipoproteins

It has been well established that atherosclerotic vascular disease is less frequent in premenopausal women than in men of similar age.[1] Although the mechanisms of action are not clear, this sex difference is thought to be related, at least in part, to effects of the sex steroids on lipid metabolism. The premenopausal lipoprotein profile that is characterized by higher high-density lipoprotein (HDL) cholesterol and lower low-density lipoprotein (LDL) cholesterol concentration compared with that of the male profile is considered antiatherogenic.[2] Moreover, oral estrogens administered to postmenopausal women have been shown to increase HDL and decrease LDL.[3]

We have previously demonstrated that surgically menopausal women who received an estrogen-androgen preparation parenterally as replacement therapy had fewer physical and psychological symptoms,[4] less depressed mood,[5] and increased levels of sexual functioning[6] compared with women who received estrogen alone. Because of the strikingly superior clinical efficacy of the combined drug compared with estrogen alone, it seemed important to determine whether the addition of androgen to a postmenopausal estrogen replacement regimen might affect lipoprotein metabolism in women thus treated. More specifically, our goal was to investigate (1) whether parenteral testosterone, administered in combination with long-term estrogen, would militate against the beneficial effects of estrogen on lipoprotein subfractions and/or (2) whether it would induce a malelike lipoprotein profile in treated women, thereby possibly increasing their risk of coronary heart disease.

From the Department of Psychology, McGill University, and the Departments of Obstetrics and Gynecology and Clinical Chemistry, The Sir Mortimer B. Davis–Jewish General Hospital, McGill University.

Supported by the Medical Research Council of Canada (Grant No. MA-8707 to B. B. Sherwin).

Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.

Reprint requests: Dr. B. B. Sherwin, McGill University, Department of Psychology, 1205 Dr. Penfield Ave., Montreal, Quebec, Canada H3A 1B1.

Material and methods

Subjects. The participants in this study were three groups of women who had undergone a total abdominal hysterectomy and bilateral salpingo-oophorectomy for benign disease at least 2 years before recruitment into the study. Women in group 1 had been receiving an estrogen-androgen preparation intramuscularly once a month since operation. Group 2 was composed of women who were given a monthly injection of estrogen alone while women in group 3 had remained untreated since total abdominal hysterectomy and bilateral salpingo-oophorectomy at least 2 years ago. Subjects in the three groups were matched for age, weight, height, and years since operation. Fuurther selection criteria required that subjects be free of acute or chronic disease and that they not be receiving any other

Table I. Subject characteristics in the experimental and control groups (mean ± SD)

	Group 1 (estrogen-androgen)	*Group 2 (estrogen alone)*	*Group 3 (control, untreated)*
Age	50.2 ± 4.7	52.1 ± 5.3	53.1 ± 5.8
Weight (pounds)	138.8 ± 7.3	135.7 ± 6.8	141.2 ± 8.1
Height (inches)	64.6 ± 2.0	63.1 ± 2.2	63.0 ± 1.7
Years since operation	3.6 ± 1.4	4.2 ± 2.1	4.9 ± 2.4

Table II. Mean plasma estradiol levels for the seven test times

	Estradiol (pg/ml) (mean ± SEM)		
Time	*Estrogen-androgen group*	*Estrogen-alone group*	*Control group*
Baseline	67.9 ± 1.7*†	33.0 ± 1.6‡	4.9 ± 1.2
Day 2	258.1 ± 5.6	363.8 ± 8.1†	—
Day 4	167.8 ± 3.3	354.3 ± 10.8†	—
Day 8	111.6 ± 2.5§	270.5 ± 21.0†	—
Day 15	103.8 ± 1.7§	175.3 ± 11.8*†	—
Day 21	90.0 ± 1.7§	116.6 ± 8.0*	—
Day 28	89.3 ± 1.8†§	66.9 ± 6.1*†	5.3 ± 1.4

*Within-group significant difference at $p < 0.01$ level compared with values for days 2, 4, and 8.
†Significantly greater than values for other group(s) at $p = 0.01$ level.
‡Within-group significant difference at $p < 0.01$ level compared with values for days 2, 4, 8, and 15.
§Within-group significant difference at $p < 0.01$ level compared with values for days 2 and 4.

medications. Women who ingested more than 6 ounces of wine or 2 ounces of liquor per day or who smoked were also excluded from the study. Subjects recruited from the McGill Menopause Clinic gave informed, signed consent by means of a form that had been approved by a university hospital ethics committee.

Procedure. A three (groups) by seven (time periods) design was used. The attempt was made to establish a baseline by which time the previous dose of hormones would have been as completely metabolized as possible. Accordingly, subjects in groups 1 and 2 were asked to delay injection of hormone(s) for 4 weeks beyond their usual monthly appointment. At the onset of the study, therefore, the women would not have received a hormone injection for approximately 56 days. Then 30 ml of blood was drawn by venipuncture for the measurement of plasma estradiol, testosterone, triglycerides, HDL, LDL, and total cholesterol. This first sample, taken approximately 56 days after hormone administration, was designated as the baseline measure. Subjects in group 1 were then injected with 1 ml of the estrogen-androgen drug they had previously been receiving and group 2 received the usual dosage (1 ml) of the estrogen-alone drug. Blood was subsequently sampled on days 2, 4, 8, 15, 21, and 28 after injection for assay of plasma hormone levels and lipoprotein subfraction concentrations. Untreated women in the control group had two blood samples taken 28 days apart for measurement of hormone and lipoprotein concentrations.

Drugs. One milliliter of the estrogen-androgen combined drug (Climacteron) and 1 ml of the estrogen-alone drug (Delestrogen) were administered intramuscularly once during the study to subjects in groups 1 and 2, respectively. These were the same drugs in the same recomended dosages as had been given to women in groups 1 and 2 monthly for approximately 2 years before this investigation. The specific constituents and dosages per milliliter of both hormonal preparations are as follows: For the estrogen-androgen combined drug (Climacteron), each milliliter contains 150.0 mg of testosterone enanthate benzilic acid hydrozone, 7.5 mg of estradiol dienanthate, and 1.0 mg of estradiol benzoate. For the estrogen-alone drug (Delestrogen), each milliliter contains 10.0 mg of estradiol valerate.

Plasma hormone levels. Thirty milliliters of blood was collected from each subject at each test time. Blood sampling occurred between 8 and 9 AM after an overnight 15-hour fast. Fifteen milliliters of blood was immediately centrifuged and the plasma was stored at −20° C for the hormone assays undertaken at the conclusion of the study. Plasma estradiol was measured in duplicate by radioimmunoassay with the use of an antibody raised to 17β-estradiol–succinyl-bovine serum albumin after extraction into ethyl ether; charcoal was used as the adsorbent.* The limit of the assay sensitivity was 5 pg/ml. Serum testosterone levels

*A list of references for all assays will be provided on request to the authors.

Table III. Mean plasma testosterone levels for the seven test times

Time	*Testosterone (ng/ml) (mean ± SEM)*		
	Estrogen-androgen group	*Estrogen-alone group*	*Control group*
Baseline	2.1 ± 0.04*†	0.38 ± 0.1	0.22 + 0.01
Day 2	2.6 ± 0.05*	0.34 ± 0.07	—
Day 4	2.7 ± 0.05*	0.42 ± 0.04	—
Day 8	3.1 ± 0.06*	0.36 ± 0.08	—
Day 15	2.7 ± 0.05*	0.32 ± 0.07	—
Day 21	2.4 ± 0.04*‡	0.30 ± 0.07	—
Day 28	2.3 ± 0.04*‡	0.28 ± 0.01	0.24 + 0.01

*Significantly greater than values for the other group(s) at p = 0.01 level.
†Within-group significant difference at $p < 0.01$ level compared with values for days 4, 8, and 15.
‡Within-group significant difference at $p < 0.01$ level compared with value for day 8.

Table IV. LDL/HDL ratios (mean ± SD) for the three groups at each test time

Time	*Group 1 (estrogen-androgen)*	*Group 2 (estrogen alone)*	*Group 3 (control, untreated)*
Baseline	3.11 ± 1.2	3.08 ± 1.6	3.15 ± 0.6
Day 2	3.16 ± 1.4	2.78 ± 1.6	—
Day 4	3.04 ± 1.3	2.54 ± 1.3	—
Day 8	2.98 ± 1.3	2.63 ± 1.1	—
Day 15	4.15 ± 1.2	2.87 ± 1.4	—
Day 21	3.04 ± 1.3	2.90 ± 1.5	—
Day 28	2.92 ± 1.3	2.96 ± 1.6	3.22 ± 0.8

were measured by means of the immuno-testosterone iodine 125 extraction method. The lower level of sensitivity of the testosterone ^{125}I assay in female serum was 0.1 ng/ml. All samples from each subject were measured twice in the same assay at the conclusion of the study.

Lipoprotein lipids. Serum triglyceride determinations were performed with the S.V.R. Enzymatic Triglycerides Tests obtained from Behring Diagnostica (La Jolla, California), based on the method of Bucolo and David. Total serum cholesterol was measured by an enzymatic technique with a reagent obtained from Fisher Scientific, Montreal, Quebec, Canada. Serum HDL cholesterol was determined according to a method of precipitation with polyanions described by Burnstein et al. LDL cholesterol was estimated by indirect calculation (LDL cholesterol = total cholesterol − [HDL + triglycerides/5]).

Statistical analyses. Hormonal and lipid data were analyzed with the use of BMDP analysis of variance for repeated measures for groups × time blocks. Within- and between-group Tukey post hoc tests were then used to probe significant group × time analysis of variance interactions when they occurred.

Results

Subject characteristics. Forty-two women participated in this study; 20 were in group 1, 11 in group 2, and 11 in group 3. As seen in Table I, there were no significant differences between the three groups for any of the matching variables we used initially. Moreover, all subjects were in good general health and none were currently receiving any medications except for hormones. Review of pathology reports of the subjects revealed that all of the women had undergone total abdominal hysterectomy and bilateral salpingo-oophorectomy for benign uterine fibroids. At the outset of the study, subjects were able to delay monthly injections for a mean of 55 days (range 52 to 55 days). Therefore, this period of time defines the baseline measure.

Plasma hormone levels. Mean plasma estradiol levels for the seven test times appear in Table II. Except for the baseline measure, estradiol values of both hormone groups exceeded those of the untreated control group at every test time ($p < 0.01$). Also, on days 2, 4, 8, and 15 after injection, women in the estrogen-alone group had mean estradiol levels that were higher than levels of those who received the combined estrogen-androgen drug ($p < 0.01$).

Analysis of plasma testosterone levels showed that values of the combined estrogen-androgen group exceeded those of both the estrogen-alone and the control group at every test time ($p < 0.01$) (Table III). Within-group analyses showed that the estrogen-androgen group's testosterone levels were greater on days 4, 8, and 15 after injection compared with baseline and postinjection day 28 values. However, testosterone levels of the estrogen-alone and the control groups remained stable throughout the course of the investigation.

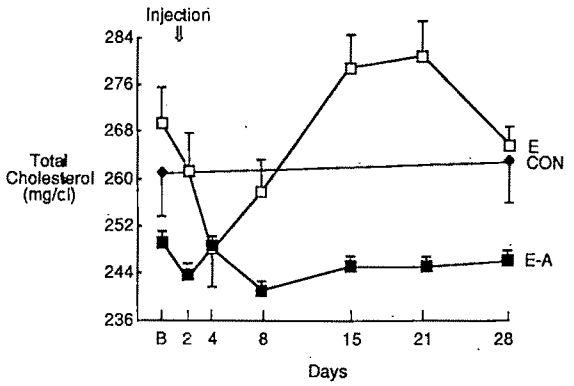


Fig. 1. Total cholesterol levels (mean ± SEM). *E* = Estrogen group; *CON* = control group; *E-A* = estrogen-androgen group.

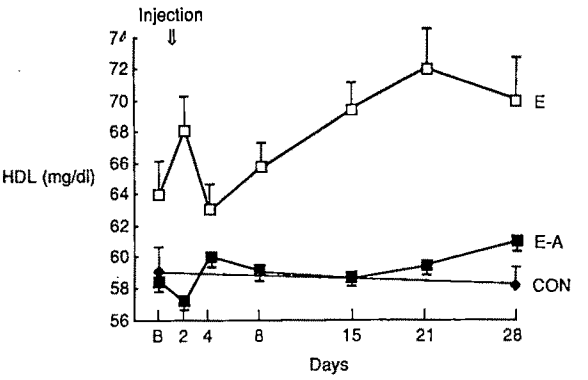


Fig. 2. HDL levels (mean ± SEM). *E* = Estrogen group; *CON* = control group; *E-A* = estrogen-androgen group.

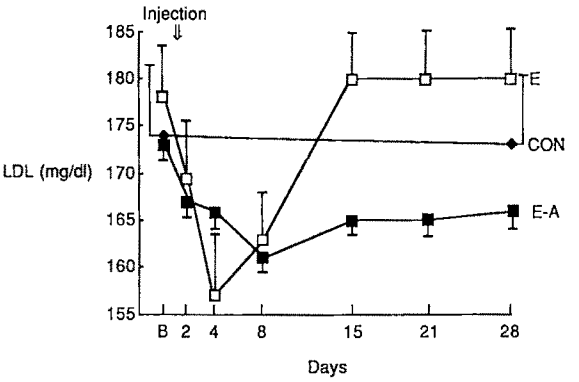


Fig. 3. LDL levels (mean ± SEM). *E* = Estrogen group; *CON* = control group; *E-A* = estrogen-androgen group.

Lipoprotein cholesterol. Total cholesterol levels of the three groups appear in Fig. 1. Although there were no significant differences between the cholesterol levels of the three groups at any of the test times, the analysis of variance did show a significant main effect for group ($p < 0.01$). Post hoc tests revealed that whereas total cholesterol levels of both the combined estrogen-androgen group and the control group remained quite stable throughout the treatment phase, values of the estrogen-alone group were lower on day 4 after injection compared with values on days 15 and 21 ($p < 0.05$). Analysis of triglyceride levels showed that there were no significant differences between the groups at any of the test times throughout the course of the study. Neither did triglycerides levels fluctuate in the two hormone-treated groups in association with changes in the circulating hormone levels as the drugs were being metabolized.

Differences in HDL levels between the groups also failed to attain statistical significance at any point in time during the study, as seen in Fig. 2. However, once again, the analysis of variance showed a main effect for group ($p < 0.02$). Within-group post hoc analyses showed that, in the combined estrogen-androgen group, HDL levels were lower on day 2 after injection than on day 28 ($p < 0.05$), and in the estrogen-alone group, HDL values were lower at baseline and on day 4 after injection than on day 21 ($p < 0.05$). Similarly, LDL levels also differed only within the two hormone-treated groups. As seen in Fig. 3, LDL was lower in the combined group on postinjection day 8 compared with baseline ($p < 0.05$). In the estrogen-alone group, LDL was lower on days 4 and 8 after injection compared with baseline values and values on days 15, 21, and 28 ($p < 0.05$).

Mean LDL/HDL ratios were computed for each group at each time period (Table IV). The analysis of variance on these data failed to find a significant interaction or main effect.

Comment

The major finding of this study was that the addition of testosterone to a parenteral estrogen replacement regimen for postmenopausal women did not adversely affect the lipoprotein cholesterol profile in these women compared with patients treated with parenteral estrogen alone and with bilaterally oophorectomized women who remained untreated. Before we discuss these results, several points need to be addressed.

The dosage of estradiol administered to the estrogen-alone group (estradiol valerate, 10 mg) and that administered to the combined estrogen-androgen group (estradiol dienanthate, 7.5 mg, and estradiol benzoate, 1 mg) both induced increases in plasma estradiol levels that remained significantly higher than baseline levels until postinjection day 15. The estrogen-alone drug induced higher estradiol levels than did the combined preparation for the first postinjection week, but at all times during the study, plasma estradiol levels were within the range of normally fluctuating menstrual cycle values in both hormone-treated groups.[7] These values also fall within the ranges that are thought to provide adequate protection against postmenopausal osteoporosis.[8] On the other hand, plasma testosterone levels of women who received the combined drug ex-

ceeded those of the estrogen-treated and the control groups at every point in time during the course of the investigation. These values also exceeded norms for ovulating women as well as the norms for women established in the laboratory that carried out these assays (0.2 to 1.2 ng/ml). It was also noted that plasma testosterone levels of the combined group were sustained at higher levels for a considerable period of time (83 days in this study). Although no other reports of the intramuscular administration of testosterone to women could be located, these findings are similar to those documented after implant therapy. In one such investigation, subcutaneous implantation of pellets containing 75 mg of estradiol and 75 mg of testosterone induced plasma testosterone levels that peaked at 2.5 ng/ml after 2 weeks.[9] Moreover, circulating testosterone levels in that study did not decrease to pretreatment values until 18 weeks after implantation. Plasma testosterone levels that exceeded female norms were also reported one month after subcutaneous implantation of 40 mg of estradiol and 100 mg of testosterone, and levels still remained elevated above the normal female limit by 6 months.[10] Our findings indicate that intramuscularly administered testosterone is also metabolized very slowly. Despite this, only one subject who received the combined drug had hirsutism that was characterized by a mild growth of hair on the chin and a moderate growth on the abdomen and legs. None of the other women had any signs of virilization as determined by direct questioning and by physical examination.

The evidence that testosterone decreases HDL and increases LDL, thereby creating an atherogenic lipid profile, is impressive. Firstly, life-span distribution of LDL and HDL in both sexes shows that HDL levels are lower in men than in women from puberty onward whereas LDL levels are higher in men than in women until menopause. Furthermore, the LDL/HDL cholesterol ratio is higher in men at all stages in the life-span although the risk ratio does narrow progressively with increasing age.[11] These sex differences in lipid profiles are thought to reflect the endogenous differences in concentrations of the sex steroids. Experimental manipulation of androgens in men supports this conclusion. Normal men who received a long-acting gonadotropin-releasing hormone analog for 10 weeks had acutely lowered plasma testosterone levels that were associated with significant increases in HDL and LDL cholesterol.[12] Conversely, male athletes who self-administered fairly large doses of anabolic-androgenic steroids both orally and intramuscularly experienced a significant decrease in HDL cholesterol and an increase in LDL cholesterol compared with the values when exogenous androgenic steroids were not being used.[13]

Several studies in women have also reported that hyperandrogenemia may result in a male pattern of lipoprotein lipid concentrations that could increase atherogenic potential. Women with polycystic ovary syndrome who had elevated testosterone levels also had lower HDL and higher LDL values compared with those of normal subjects.[14] When a normolipidemic postmenopausal woman was given an androgenic, anabolic steroid orally, HDL decreased while LDL increased.[15]

On the basis of the evidence of the effects of androgen on lipoprotein lipid concentrations, it might have been predicted that our subjects who had been receiving long-term estrogen and testosterone would have had lower HDL and higher LDL values compared with values of both estrogen-treated and untreated women, yet they did not. Several explanations of our findings are possible. First, it has by now been firmly established that the administration of exogenous estrogen to postmenopausal women increases HDL and decreases LDL.[2] The findings that HDL levels of the estrogen-alone group were significantly higher by day 21 after injection and that LDL levels were lower on days 4 and 8 compared with baseline values are consistent with previous reports. It is possible therefore that the specific doses of each of the sex steroids contained in the combined preparation we used exerted an equal and opposite effect on lipoprotein lipid concentrations such that the net effect was to maintain their stability. Alternatively, it is possible that between-group differences were not significant because of the greater variability in the estrogen-alone group that, in turn, was likely due to its smaller sample size. Confirmation of these findings therefore awaits replication with larger and equal sample sizes.

A third possible explanation of our findings is based on the suggestion that the route of administration may modulate responses to hormone therapy. Percutaneous and vaginal administration of estradiol do not cause the increases in triglycerides and very low–density lipoprotein observed during oral therapy.[16, 17] In the present study as well, intramuscular injection of estradiol did not induce changes in lipoprotein lipids compared with control values of untreated women. Furthermore, it has been shown that subcutaneous implantation of pellets containing 40 mg of estradiol and 100 mg of testosterone did not cause any changes in cholesterol, triglycerides, or HDL from pretreatment levels.[10] Farish et al.[18] likewise found that subcutaneous pellets of 50 mg of estradiol and 100 mg of testosterone had no effect on HDL fractions but testosterone appeared to slightly enhance the LDL cholesterol–lowering effect of estradiol as it seemed to do in the present study.

Despite the fact that no significant between-group differences were found, there are hints in these data that the exogenous testosterone may, at times, have induced a more malelike lipoprotein profile that was of short duration. For example, the depression in HDL

levels on day 2 after injection in the combined group may have reflected a transient androgenic effect. Also, in absolute terms, HDL levels were lower in the combined group compared with the estrogen group although this fell short of statistical significance. Finally, mean LDL/HDL ratios of the combined group were higher than those of the estrogen group at six of the seven test times although, once again, these too failed to attain statistical significance. Thus, if the exogenous testosterone we administered did have an effect on lipoprotein lipids, it was mild, transient, and of questionable clinical meaningfulness.

In summary, this study found that the addition of testosterone to a parenteral estrogen replacement regimen did not adversely influence the lipid profile of postmenopausal women thus treated for 2 years. This conclusion, however, applies to the specific doses and route of administration used in this study and cannot be generalized to other androgenic compounds or to other routes of administration.

REFERENCES

1. Kannell WB, Gordon T, eds. The Framingham study. An epidemiological investigation of cardiovascular disease. Bethesda, Maryland: National Institutes of Health, 1974; NIH publication no. 74-599.
2. Gordon T, Castelli WB, Hjortland MD, Kannell WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham study. Am J Med 1977;62:707.
3. Wahl PW, Wolden CE, Knopp RH, et al. Lipid and lipoprotein triglyceride and cholesterol interrelationships: effects of sex hormone use, and hyperlipidemia. Metabolism 1984;33:502.
4. Sherwin BB, Gelfand MM. Differential symptom response to parenteral estrogen and/or androgen administration in the surgical menopause. AM J OBSTET GYNECOL 1985;151:153.
5. Sherwin BB, Gelfand MM. Sex steroids and affect in the surgical menopause: a double-blind, cross-over study. Psychoneuroendocrinology 1985;10:325.
6. Sherwin BB, Gelfand MM, Brender W. Androgen enhances sexual motivation in females: a prospective, cross-over study of sex steroid administration in the surgical menopause. Psychosom Med 1985;47:339.
7. Mishell DR, Nakamura RM, Crosignani PG, et al. Steroid gonadotropin and steroid patterns during the normal menstrual cycle. AM J OBSTET GYNECOL 1971;111:60.
8. Gordon GS. Prevention of bone loss and fractures in women. Maturitas 1984;6:225.
9. Kapentanakis E, Dmowski WP, Auletta F, Scommenga A. Endocrine and clinical effects of estradiol and testosterone pellets used in long-term replacement therapy. Int J Gynaecol Obstet 1982;20:387.
10. Burger HG, Hailes J, Menelaus M, Nelson J, Hudson B, Balazo W. The management of persistent menopausal symptoms with oestradiol-testosterone implants: clincial lipid and hormonal results. Maturitas 1984;6:351.
11. Hazzard WR. The sex differential in longevity. In: Andres RA, Bierman EL, Hazzard WR, eds. Principles of geriatric medicine. New York: McGraw-Hill, 1984:137.
12. Goldberg RB, Rabin D, Alexander AN, Doelle GC, Getz GS. Suppression of plasma testosterone leads to an increase in serum total and high density lipoprotein cholesterol and apoproteins A-I and B. J Clin Endocrinol Metab 1985;60:203.
13. Webb OL, Laskarzewski PM, Glueck CJ. Severe depression of high-density lipoprotein cholesterol levels in weight lifters and body builders by self-administered exogenous testosterone and anabolic-androgenic steroids. Metabolism 1984;33:971.
14. Wild RA, Painter PC, Coulson PB, Carruth KB, Ravney GB. Lipoprotein lipid concentrations and cardiovascular risk in women with polycystic ovary syndrome. J Clin Endocrinol Metab 1985;61:946.
15. Hazzard WR, Hoffner SM, Kushwaha RS, Applebaum-Bowden D, Foster DM. Preliminary report: kinetic studies on the modulation of high-density lipoprotein alolipoprotein, and subfraction metabolism by sex steroids in a postmenopausal woman. Metabolism 1984;33:779.
16. Mishell DR Jr, Moore DE, Roy S, Brenner PF, Page MA. Clinical performance and endocrine profiles with contraceptive vaginal rings containing a combination of estradiol and *d*-norgestel. AM J OBSTET GYNECOL 1978;130:55.
17. Whitehead MI, Townsend PT, Kitchin Y, et al. Plasma steroid and protein hormone profiles in postmenopausal women following topical administration of oestradiol 17β. In: Mauvais-Jarvis P, Vickers GFH, Wepierre J, eds. Percutaneous absorption of steroids. New York: Academic Press, 1980:231.
18. Farish E, Fletcher CD, Hart DM, Azzawi EA, Abdalla HI, Gray CE. The effect of hormone implants on serum lipoproteins and steroid hormones in bilaterally oophorectomized women. Acta Endocrinol 1984;106:116.

Effects of adenosine 3′,5′-cyclic monophosphate, forskolin, and cholera toxin on hormone production in human term placental cells

Fuzhen Zhou,* Basil Ho Yuen, M.B., and Peter C. K. Leung, Ph.D.**
Vancouver, British Columbia, Canada

Trophoblast cells from human term placenta in monolayer culture were used to investigate the role of adenosine 3′,5′-cyclic monophosphate in the production of human chorionic gonadotropin and estradiol-17β. The intracellular concentration of adenosine 3′,5′-cyclic monophosphate was elevated by (1) addition of an adenosine 3′,5′-cyclic monophosphate analogue, 8-bromoadenosine 3′,5′-cyclic monophosphate, (2) inhibition of the hydrolysis of G_s-GTP complex by cholera toxin, and (3) direct stimulation of adenylate cyclase with forskolin. Addition of 2 mmol/L of 8-bromoadenosine 3′,5′-cyclic monophosphate markedly increased the accumulation of human chorionic gonadotropin in the culture medium on days 2, 3, and 4 of treatment. Likewise, addition of cholera toxin (0.2 μg/ml) or forskolin (50 μmol/L) also enhanced human chorionic gonadotropin production. On the other hand, the production of estradiol-17β was significantly inhibited by 8-bromoadenosine 3′,5′-cyclic monophosphate, at the same time that human chorionic gonadotropin production was enhanced in the same experiments. These results further support a differential role of adenosine 3′,5′-cyclic monophosphate on human chorionic gonadotropin and estradiol-17β production in the human term placenta. (Am J Obstet Gynecol 1987;156:420-4.)

Key words: Chorionic gonadotropin, human, placental cell culture, cyclic adenosine monophosphate

Although various factors that influence placental hormone production have been studied extensively,[1] controversies exist concerning the possible role of these factors. With regard to human chorionic gonadotropin (hCG), it has been shown that the production of hCG by trophoblast is stimulated by adenosine 3′,5′-cyclic monophosphate (cAMP).[2, 3] To our knowledge, the effects of other factors that perturb the cAMP-generating system at the cellular level (such as forskolin and cholera toxin) on hCG have not been investigated. The effects of cAMP on placental steroidogenesis appear to be more complex. While stimulation of trophoblast aromatase activity by cAMP has been demonstrated in choriocarcinoma cells,[2-4] cAMP inhibited estradiol-17β secretion in human term placental explants supplemented with dehydroepiandrosterone sulfate.[5] On the other hand, other investigators have shown that the addition of dibutyryl cAMP plus theophylline to the culture medium had no effect on aromatase activity 24 hours after its addition but decreased aromatase activity after 48 hours in placental cell culture.[6]

In view of the still confusing role of cAMP on placental hormone production, the purpose of this study is to examine the effects of exogenous cAMP, as well as forskolin and cholera toxin, on the production of hCG and estradiol-17β, with use of human term placental cells in monolayer culture.

Material and methods

Cell culture. The placental cells were prepared according to Hall et al.[7] and Branchaud et al.[8] with some modifications. The portions of term placental cotyledons obtained from cesarean section were transported in Hanks balanced salt solution without calcium and magnesium (Gibco) under aseptic conditions. The tissues were rinsed thoroughly with Hanks balanced salt solution and finely minced. The excess buffer was discarded following centrifugation at 50 × g for 5 minutes. Approximately 5 to 10 gm of tissue was then digested in 30 to 100 ml of 0.25% trypsin (Gibco) and deoxyribonuclease (500 U/100 ml, Sigma) in Hanks balanced salt solution. The digest was incubated at 37° C and agitated by hand intermittently every 5 minutes. After 10 to 15 minutes of incubation the supernatant

From the Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of British Columbia, Grace Hospital.
Presented at the Forty-second Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Charlottetown, Prince Edward Island, Canada, June 23-27, 1986.
Reprint requests: Dr. Peter C. K. Leung, Department of Obstetrics and Gynecology, Grace Hospital, 4490 Oak St., Vancouver, British Columbia, Canada V6H 3V5.
**Fellow of the World Health Organization.*
***Recipient of a Medical Research Council Scholar Award.*

Table I. Effects of 8-Br-cAMP on hCG and estradiol-17β production in human placental cells in monolayer culture

Treatment	*Day of treatment*			
	1	*2*	*3*	*4*
hCG (mIU/ml)				
Control	6.5 ± 0.6	62.8 ± 3.9	230.8 ± 14.7	265.8 ± 20.8
8-Br-cAMP		123.3 ± 13.6*	280.5 ± 17.2†	427.8 ± 18.4*
Estradiol-17β (pg/ml)				
Control	340 ± 38	552 ± 67	582 ± 39	597 ± 48
8-Br-cAMP		364 ± 29*	346 ± 23†	373 ± 62†

*$p < 0.01$.
†$p < 0.05$.

Table II. Effects of forskolin on hCG and estradiol-17β production on human placental cells in monolayer culture

Treatment	*Day of treatment*			
	1	*2*	*3*	*4*
hCG (mIU/ml)				
Control	7.2 ± 0.5	19.5 ± 0.5	113.3 ± 8.2	71.4 ± 15.7
Forskolin		31.4 ± 3.2*	218.1 ± 8.1*	192.7 ± 40.8†
Estradiol-17β (pg/ml)				
Control	506 ± 25	643 ± 35	722 ± 80	672 ± 31
Forskolin		591 ± 48	647 ± 64	653 ± 29

*$p < 0.01$.
†$p < 0.05$.

was transferred to centrifuge tubes containing 0.7 ml of fetal bovine serum (Gibco). The cells were harvested by centrifugation at 50 × g for 10 minutes. The supernatant was discarded. The pellet was covered by fetal bovine serum and kept in ice bath. This procedure was repeated up to six times. The large amount of erythrocytes was lysed by adding 10 ml of freshly made ammonium chloride–phosphate buffer (0.83% ammonium chloride in phosphate-buffered saline solution, pH 7.2) and kept in an ice bath for 8 minutes. One ml of fetal bovine serum was then layered onto the bottom of the tube carefully. The tube was centrifuged at 100 × g for 8 minutes. The pellet was washed once and resuspended in minimal essential medium (Gibco) with 10% fetal bovine serum. Aliquots of approximately 5×10^5 cells/ml were plated in 24-well cultured plates (Falcon) and incubated at 37° C in an atmosphere of 95% air and 5% carbon dioxide for 4 days. The culture medium was changed daily. The cells were treated with 8-bromoadenosine 3′,5′-cyclic monophosphate (8-Br-cAMP, 2 mmol/L, Sigma), forskolin (50 μmol/L, Sigma) or cholera toxin (200 ng/ml, Sigma). At the end of the experiment the culture medium samples were stored at −20° C until hormone measurements.

All experiments were performed in quadruplicate. Each sample of culture medium was assayed for hCG and estradiol-17β by radioimmunoassays, as previously described.[9] Measurement of hCG was made with the Amerlex β-hCG radioimmunoassay kits purchased from Amersham. Data for hCG are based on the second international standard. Results are presented as the mean ± SE. Statistical differences were determined by analysis of variance.

Results

As indicated in Tables I and II, basal production of hCG by the cultured placental cells increased markedly in a linear fashion from the low levels observed on day 1 of treatment (6.5 ± 0.6 mIU/ml and 7.2 ± 0.5 mIU/ml, respectively) to reach maximal hCG levels on day 3 (230 ± 14 mIU/ml and 113 ± 8 mIU/ml, respectively). This represents a 15- to 35-fold increase in hCG production in the control cultures over a period of 3 days. By contrast, the production of basal estradiol-17β in the same experiments was already maximal on the first day so that the levels of estradiol-17β in the control cultures were not significantly altered ($p > 0.05$) on days 2, 3, and 4.

Addition of 2 mmol/L of 8-Br-cAMP to the cultured placental cells markedly enhanced hCG production (Table I). The stimulation of hCG was already significant ($p < 0.01$) as early as 24 hours after 8-Br-cAMP addition, with the level of hCG at this time reaching

Table III. Effects of forskolin and cholera toxin on hCG and estradiol-17β secretion by human placental cells in monolayer culture

Treatment	*hCG (mIU/ml)*	*Estradiol-17β (pg/ml)*
Control	72.8 ± 5	514 ± 30
Forskolin	171.7 ± 11.1*	548 ± 53
Cholera toxin	124.9 ± 6.1†	560 ± 115

*$p < 0.01$.
†$p < 0.05$.

200% of that in control cultures. The stimulated level of hCG remained linear up to day 4 of treatment. On day 4 of 8-Br-cAMP stimulation the level of hCG was 160% of that observed in control cultures ($p < 0.01$). Similar results were obtained in at least three other separate experiments.

In sharp contrast to the stimulatory action on hCG production, the addition of 2 mmol/L of 8-Br-cAMP significantly attenuated estradiol-17β production in the same cultured placental cells. As indicated also in Table I, the inhibitory effect on estradiol-17β was observed as early as 24 hours after addition of 8-Br-cAMP (from 552 ± 67 pg/ml to 364 ± 27 pg/ml, $p < 0.05$). On day 4 of treatment the level of estradiol-17β in the 8-Br-cAMP–treated cultures was still significantly suppressed ($p < 0.01$) to 62% of the level observed in the control cultures.

Since forskolin activates adenylate cyclase directly to stimulate intracellular production of cAMP, we have examined the possible effect of forskolin on placental hCG and estradiol-17β production. It can be seen in Table II that 50 μmol/L forskolin, a dose previously shown to stimulate cAMP production in other tissues, markedly enhanced hCG production in cultured placental cells. Like 8-Br-cAMP, the stimulatory effect of forskolin was observed as early as 1 day after its addition to the cells ($p < 0.01$). On day 4, the forskolin-stimulated hCG level was 270% when compared with that found in control cultures ($p < 0.01$). Also, the addition of 10 μmol/L of forskolin was effective in stimulating hCG production in other experiments (not shown). On the other hand, addition of either 10 or 50 μmol/L of forskolin failed to significantly affect the levels of estradiol-17β on days 2, 3, or 4 of treatment ($p > 0.05$, Table II).

We next examined the possible effects of cholera toxin, which is also known to stimulate cAMP production. As in the previous experiments with forskolin, addition of cholera toxin to the culture medium (on day 1) also stimulated hCG production on days 2, 3, and 4 of culture. Results of a representative experiment are shown in Table III. Addition of cholera toxin (200 ng/ml) on day 1 caused a significant increase (170%) in hCG ($p < 0.01$) when compared with control cultures on day 2 of treatment. In the same experiment, 50 μmol/L of forskolin was slightly more effective than cholera toxin, stimulating hCG to 237% of control level on day 2 ($p < 0.01$). The degrees of hCG stimulation caused by cholera toxin and forskolin, however, were the same on days 3 and 4 of treatment (not shown). Like forskolin, the presence of cholera toxin also did not affect the production of estradiol-17β when compared with control cultures (Table III). Again, almost identical results were obtained in at least two other experiments.

Comment

The present results are consistent with previous reports that the production of hCG in trophoblast cells is stimulated by cAMP.[2, 3, 10] In this study the intracellular concentrations of cAMP is elevated by (1) the addition of a cAMP derivative, 8-bromo-cyclic AMP, (2) direct stimulation of adenylate cyclase with forskolin, or (3) inhibition of the hydrolysis of G_s-GTP complex by cholera toxin. In the present placental cell culture system, the basal hCG level reaches its peak value on day 3 and remained elevated on day 4 of the experiment. Addition of 8-Br-cAMP, forskolin, or cholera toxin markedly stimulated hCG production. This stimulation could be observed as early as 24 hours after treatment and persisted at least to the end of the study on the fourth day.

The role of cAMP in the regulation of hCG production is not clear. Khodr and colleagues[11, 12] have proposed that placental hormonal production could be affected by human chorionic gonadotropin–releasing hormone (hCGnRH) which is synthesized by cytotrophoblast. The stimulatory action of hCGnRH on hCG is believed to be cAMP-mediated. In support of this, a gonadotropin-releasing hormone receptor that activates cAMP has been reported in human placenta by Currie et al.[13] Also, it has been demonstrated that cAMP itself is accumulated after treatment of placenta with exogenous gonadotropin-releasing hormone. Thus the present finding of remarkably similar stimulation of hCG by 8-Br-cAMP, forskolin, and cholera toxin may well reflect a role of cAMP in the signal transduction of hCGnRH in the human placenta.

The influence of cAMP on placental estradiol-17β production is more controversial. In choriocarcinoma cells aromatase activity could be stimulated by cAMP.[2-4] Moreover, Hochberg et al.[14] reported that addition of dibutyryl cAMP to placental cell culture stimulated estrogen secretion. By contrast, Lobo et al.[6] failed to stimulate aromatase activity with the combination of cAMP and theophylline in human term pla-

cental cell in monolayer culture, while Haning et al.[5] reported that cAMP decreased estradiol-17β output in placental explants supplemented with dehydroepiandrosterone sulfate.

Unlike hCG, the production of estradiol-17β remained relatively unchanged throughout the 4 days of experiment in the present study. As no additional precursor steroids (for example, dehydroepiandrosterone sulfate) were added to the culture medium, estradiol-17β production by the placental cells was low and probably dependent on the very low concentrations of precursor steroids in the fetal bovine serum added. Nevertheless, in most experiments the concentration of estradiol-17β in the culture medium reached its peak value by day 2 and remained elevated above day 1 levels until the end of the study (that is, day 4). In contrast to its effect on hCG, the addition of 8-Br-cAMP to the placental cells caused a significant inhibition of estradiol-17β production. This latter effect occurred as early as 24 hours after the addition of 8-Br-cAMP and persisted throughout the entire 3 days of treatment. In placental explants, other investigators have proposed that the inhibitory effect of 8-Br-cAMP on estradiol-17β production may result from a possible blockade of steroid 3-sulfatase, the rate-limiting step of conversion of dehydroepiandrosterone sulfate to estradiol-17β in trophoblast cells.[5] In the present study, since the steroid precursors available to the placental cells from the fetal bovine serum were presumably low in concentration and certainly not all sulfated, the precise locus of inhibition caused by 8-Br-cAMP remains to be determined. Likewise, the failure of either forskolin or cholera toxin to affect estradiol-17β production is rather difficult to explain, especially in view of the present finding that both forskolin and cholera toxin were effective in stimulating hCG production in the same placental cells. On the assumption that steroid 3-sulfatase or aromatase is indeed affected by cAMP, the possibility exists that the intracellular concentrations of cAMP induced by forskolin or CT, while effective in enhancing hCG production, may not be sufficient enough to significantly perturb these enzymes in the placental cells. Further investigation is warranted to clarify this point.

More recently Siler-Khodr et al.[12, 15] have shown that, while synthetic gonadotropin-releasing hormone stimulates both hCG and estradiol-17β release from term placental tissue culture, the stimulation of hCG production by gonadotropin-releasing hormone only occurred after extended culture and when steroid levels were low. Likewise, Branchaud et al.[8] have found that the addition of dehydroepiandrosterone sulfate to the culture medium of placental cell cultures significantly inhibited the stimulatory action of gonadotropin-releasing hormone in hCG release. In the present study, 2 mmol/L of 8-Br-cAMP stimulated hCG release, while the production of estradiol-17β was inhibited in the same cultures. Thus it can be speculated that the modulation of placental production of hCG and estradiol-17β by cAMP, like gonadotropin-releasing hormone, may follow a reciprocal relationship. Further investigations will be needed to determine which specific enzymes are altered by elevated cAMP concentrations, as well as the possible causal relationship between gonadotropin-releasing hormone and cAMP action in the placental cells.

The excellent technical and editorial assistance of Ms. Joan Morgan and Miss Mary-Ann Ungless is gratefully acknowledged.

REFERENCES

1. Krieger DT. Placenta as a source of 'brain' and 'pituitary' hormones. Biol Reprod 1982;26:57.
2. Hussa RO, Story MT, Pattillo RA. Cyclic adenosine monophosphate stimulates secretion of human chorionic gonadotropin and estrogens by human trophoblast in vitro. J Clin Endocrinol Metab 1974;38:338.
3. Story MT, Hussa RO, Pattillo RA. Independent dibutyryl cyclic adenosine monophosphate stimulation of human chorionic gonadotropin and estrogen secretion by malignant trophoblast cells in vitro. J Clin Endocrinol Metab 1974;39:877.
4. Bellino FL, Hussa RO, Osawa Y. Estrogen synthetase in choriocarcinoma cell culture: stimulation by dibutyryl cyclic adenosine monophosphate and theophylline. Steroids 1978;32:37.
5. Haning RV Jr, Choi L, Kiggens AJ, Kuzma DL, Summerville JW. Effects of dibutyryl adenosine 3′,5′-monophosphate, luteinizing hormone-releasing hormone, and aromatase inhibitor on simultaneous outputs of progesterone, 17β-estradiol, and human chorionic gonadotropin by term placental explants. J Clin Endocrinol Metab 1982;55:213.
6. Lobo JO, Bellino FL, Bankert L. Estrogen synthetase activity in human term placental cells in monolayer culture. Endocrinology 1985;116:889.
7. Hall CSG, James TE, Goodyer C, Branchaud C, Guyda H, Giroud CJP. Short term tissue culture of human midterm and term placenta: parameters of hormonogenesis. Steroids 1977;30:569.
8. Branchaud CL, Goodyer CG, Lipowski LS. Progesterone and estrogen production by placental monolayer cultures: effects of dehydroepiandrosterone and luteinizing hormone-releasing hormone. J Clin Endocrinol Metab 1983; 56:761.
9. Leung PCK, Goldenberg SR, Armstrong DT. Changes with age in aromatization of testosterone in immature female rats. Biol Reprod 1978;19:1036.
10. Hussa RO, Pattillo RA, Ruckert ACF, Scheuermann KW. Effects of butyrate and dibutyryl cyclic AMP on hCG-secreting trophoblastic and non-trophoblastic cells. J Clin Endocrinol Metab 1978;46:69.
11. Khodr GS, Siler-Khodr TM. Placental luteinizing hormone-releasing factor and its synthesis. Science 1980; 207:315.
12. Siler-Khodr TM, Khodr GS, Valenzuela G, Rhode J. Gonadotropin-releasing hormone effects on placental hormones during gestation. I. Alpha-human chorionic gonadotropin, human chorionic gonadotropin and human chorionic somatomammotrophin. Biol Reprod 1986;34: 245.

13. Currie AJ, Fraser HM, Sharpe RM. Human placental receptors for luteinizing hormone-releasing hormone. Biochem Biophys Res Commun 1981;99:332.
14. Hochberg Z, Perlman R, Bendeoli A, Braudes JM. The effect of glucose, 2-deoxy-D-glucose and insulin on estradiol secretion by cultured human trophoblast. Biochem Biophys Res Commun 1982;108:102.
15. Siler-Khodr TM, Khodr GS, Valenzuela G, Rhode J. Gonadotropin-releasing hormone effects on placental hormones during gestation. II. Progesterone, estrone, estradiol and estriol. Biol Reprod 1986;34:255.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1987 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 156 (January-June) and Vol. 157 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Clinical Opinion

Psychosis and pregnancy: Some new ethical and legal dilemmas for the physician

E. Earlene Dal Pozzo, M.D., and Frank H. Marsh, J.D., Ph.D.
Denver, Colorado

Physicians who treat women of childbearing age may encounter the presentation of psychosis in a pregnant woman that presents special problems in diagnosis and treatment, particularly ethical and legal considerations that center on the rights of the mother as well as the continually evolving definition of fetal rights. This article represents a collaborative effort to address these considerations. Representative case material from the treatment of a psychotic pregnant patient is presented with a focus on the nature of the contractual relationship of the treating physician with the mother and fetus. (AM J OBSTET GYNECOL 1987;156:425-7.)

Key words: Privacy, compelling interest, fetal viability, best interests, predicability of harm

Caring for a psychotic pregnant woman throughout her pregnancy presents special problems in diagnosis and treatment. Intrinsic to the treatment are compelling social, ethical, and legal considerations that center on the legal rights of the mother as well as the continually evolving definition of fetal rights. A central issue in the treatment relationship with a psychotic pregnant patient is whether that treatment relationship specifically and contractually includes the unborn fetus. If so, the treatment relationship may present areas of conflict between the expectations or rights of the woman and the potential duties owed to the fetus. Potential conflicts become apparent in those situations where the physician's duty to the fetus interferes with the mother's ability to exercise her right to privacy or other rights within the health care system and society.

In recent years the courts have distinguished a line of duty that runs directly from the physician to the unconceived and unborn child from that duty that runs to the mother (*Curlender v Bio-Sciences Laboratories* 106 p3d 814 [Cal App 1980]).[1] The rationale of these decisions is directly applicable to the relationship between the physician and pregnant woman and sets the stage for what is ethically and legally required of the physician. The following case report demonstrates the potential conflicting duties between the physician, the psychotic pregnant woman, and the fetus.

From the Consultation Liaison Division and Program in Medical Ethics and Medicine Jurisprudence, the University of Colorado School of Medicine.
Reprint requests: Frank H. Marsh, J.D., Ph.D., Program in Medical Ethics and Medicine Jurisprudence, University of Colorado School of Medicine, Box C-245, 4200 E. Ninth Ave., Denver, CO 80262.

Case report

Ms. A, a 41-year-old single, white woman, was a former boarding home resident with a long history of recurrent psychiatric hospitalizations due to chronic schizophrenia. She was brought to the emergency room on a 72-hour mental health hold after she had been found huddled in a corner of her apartment with her schizophrenic boyfriend. They were surrounded by garbage and without food.

On physical examination she was found to be approximately 27 weeks pregnant. She had received no prenatal care. She repeatedly denied her pregnancy.

On the psychiatric unit she was agitated and uncooperative. She frequently paced the ward wearing a heavy coat, mumbling to herself. She complained of hearing "demons in the night."

The patient had not taken her neuroleptic medication for several months. She accepted low-dose neuroleptic medication on the unit and returned to her usual level of functioning, that is, calm but psychotic. She no longer denied her pregnancy, but she continued to experience auditory hallucinations and delusions about the role of God in her pregnancy. She specifically denied suicidal and homicidal ideation and any thoughts of harming her baby. She asked to be discharged to return to her boyfriend's apartment. At this point in previous hospitalizations, she would have been discharged to a boarding home.

During this hospitalization, a civil commitment was requested for 90 days despite the patient's request to

leave the hospital. The commitment was requested in order to prevent any possible harm to the fetus that might result from recurrence of denial or psychotic interpretation of symptoms or through the neglect of basic nutritional and medical needs.

After 11 weeks of inpatient care she was delivered of a normal infant without complications. She decided to relinquish the infant. She was discharged to a boarding home and the civil commitment was dropped.

The physician-fetus relationship

A critical legal decision related to the definition of the treatment relationship with the pregnant patient and fetus is the 1973 landmark abortion case of *Roe v Wade* (410 US 113 163, US 1973). The Supreme Court held that a pregnant woman has a constitutional right of privacy that permits her to end her pregnancy through abortion. However, the decision to terminate a pregnancy is reserved only for the woman and her physician "free of any interference by the state" up to the point of fetal viability, at which time the state may have a "compelling interest" in both the mother's life and that of the unborn fetus. In its deliberations the court reached the following conclusion regarding the state's interest: "With respect to the state's important and legitimate interest in potential life, the 'compelling' interest is at viability . . .."

Contributing to the issues surrounding fetal viability are the immense technologic achievements during the past few years in fetal medicine that have increased survival of premature infants to earlier and earlier gestational age.[2] These developments further complicate the determination of a point of fetal viability. Traditionally, in medicine the pregnant woman and fetus have been treated as a single unit or patient. In the *Roe v Wade* decision, a separate and "compelling" interest in the fetus is described by the court.

There is substantial legal precedent in support of a preconception and/or prebirth relationship of duty between a physician and a newborn infant or fetus (*Park v Chessin*, 60 A2d 80 [NY 1977]; *Renslow v Mennonite Hospital*, 367 NE2d 1250 [IL 1977]). The fact that a physician's negligence in failing to act occurred before a child's birth does not prevent recovery for prenatal injuries or for wrongful death of a fetus.

Additional cases address the response or intervention required of the physician by the state where the life or welfare of a viable fetus is at stake. The courts, as far back as 1964, held that harm to unborn children can be adjudicated through dependency and neglect proceedings. In the New Jersey case of *Raleigh Fitkin–Paul Morgan Memorial Hospital v Anderson* (201 A2d 537 [NJ 1964], *cert denied* 377 US 985 [1964]), the state intervened under a court order to require a pregnant woman to undergo blood transfusions, to save not only her life but the life of her unborn child as well. In the more recent case of *Jefferson v Griffin Spalding County Hospital Authority*, 274 SE2d 457 (GA 1981), a court order was obtained by the state to require cesarean section over the objections of the mother where the unborn child was suffering from fetal distress. From these cases, it is fairly clear that a mother unwilling to permit life-saving medical care for a viable fetus is potentially subject to state action. The extended implications of these holdings, which are critical to the physician caring for the psychotic pregnant woman, are that intervention may be required of the physician by the state after the first trimester and most certainly at a reasonable point of viability.

A physician treating a psychotic pregnant woman has a dual line of duty: one to the pregnant patient and one to the fetus. Each treatment relationship carries a twofold responsibility: (1) to perform and (2) to do so in an acceptable manner. With this dual mother-fetus treatment relationship, three moral and legal dictates form the core of the physician's duty to perform: (1) He will be competent and will perform in a competent manner; (2) this performance will always be within the guidelines of "best interests" as expressed through the minimization of harm; (3) the patient will not be abandoned.

While the first dictate, that of professional competency, is very important, particularly in malpractice law, it is not directly involved in the present discussion. It is the second and third dictates that bear directly on the treatment relationship between the physician, the psychotic pregnant woman, and the unborn fetus and in the end will require a response from him.

It is the dictate of the "maximization of good" and the "minimization of harm" that makes caring for the psychotic pregnant woman so difficult. The physician must walk a tightrope between the respective needs and "best interests" of the fetus and those of the psychotic woman. He is expected, on the one hand, to take those steps necessary, including but not limited to invoking state action, for the protection of the viable fetus. On the other hand, if he confines or retains in custodial care a person not dangerous to herself or another and who is capable of living safely in an available community alternative, he may be held liable for civil damages.[3] This situation is compounded both by the necessity of discerning what is an unreasonable risk to the fetus and by the seemingly inequitable fact that a nonpsychotic pregnant woman who may present a comparable risk to her fetus (for instance, use of cigarettes, use of drugs, neglect of basic nutritional and medical needs) would not be held or committed to any ward or institution until she gives birth to her baby.

The extent of intervention required when a risk to the welfare of a fetus has been determined has been

interpreted as ranging from civil commitment of the pregnant psychotic woman (*O'Connor v Donaldson,* 422 US 563 [US 1975]) to reporting the case to state authorities for dependency and neglect proceedings.[3] Anything less exposes the physician to possible criminal sanctions, not to mention extensive civil liability, for failure to act where a neglected or abused unborn child is involved.

There is no easy answer to this dilemma and any resolution remains a judgment call by the physician related to "maximization of good" and the "predictability of harm." It is quite possible, because of the increasing parameters of fetal viability, that a psychotic pregnant woman, as indicated in our case report, might be held for an ever-increasing period until term. Given a logical extension of this situation, it is also quite possible in the not too distant future that "normal" pregnant mothers will likewise face hospital incarceration in those cases involving nutrition and medical neglect.

REFERENCES

1. Marsh F. Prenatal screening and "wrongful life": medicine's new "Catch-22"? Am J Obstet Gynecol 1982;143: 745-8.
2. Koops BL, et al. Neonatal mortality risk in relations to birth weight and gestational age: Update. J Pediatr 1982;101: 969-77.
3. Soloff P, Jewell S, Roth LH. Civil commitment and the rights of the unborn. Am J Psychiatry 1979;136:114-5.

Characteristics of menopausal women seeking assistance

Evelyn Anderson, R.N., Ph.D., Sonia Hamburger, B.A., James H. Liu, M.D., and Robert W. Rebar, M.D.
La Jolla, California

Using a questionnaire approach, we have determined the demographic, social, and clinical characteristics of the first 100 participants attending our menopause clinic. Of the respondents, 79% reported onset of significant physical symptoms and 63% significant emotional symptoms during the menopause. Among the participants, 65% had varying degrees of depression as determined by the Zung self-rating depression scale. This seemed to be more prevalent in patients with previous pelvic operations. Only half the women were on a regimen of estrogen replacement therapy, and most were receiving estrogen in an unopposed fashion. In our menopause clinic, more than half the women were over their ideal body weight, which is in contrast to the popular misconception that only thin women develop menopausal symptoms. Data from our patients suggest the need for multidisciplinary menopause clinics to adequately address the physical and emotional problems of menopausal women. (Am J Obstet Gynecol 1987;156:428-33.)

Key words: Menopause, estrogen replacement, depression

Menopause is of increasing importance to health professionals in terms of both preventive and clinical care of women in their later years. With the increase in life expectancy, the average woman now lives approximately one third of her life in the postmenopausal period. The rapid emergence of large numbers of menopausal women during the last several years has created the sudden need for information about the special medical problems of this group and the most appropriate ways to treat problems unique to the menopause.[1, 2]

We perceived a need to focus on the menopause as an area where sympathetic understanding, dissemination of information, and education of patients and health professionals would help women make decisions about hormonal replacement therapy and the quality of life during the transition to a mature life-style. In 1982, we conducted a 1-day symposium for lay individuals sponsored by the Department of Reproductive Medicine, University of California San Diego, School of Medicine, in an effort to determine what kind of assistance menopausal women required. Topics covered during the symposium included both physiologic and psychological aspects associated with the menopause. A questionnaire completed by 169 of the more than 200 participants strongly supported development of a clinic dedicated to the management of the menopause. Furthermore, responses favored development of a clinic along the lines of the model proposed by Utian.[1] To that end, we started a multidisciplinary outpatient service consisting of group discussions with four to six women, followed by personal counseling with each patient during the 4-hour clinic visit. Our findings with the first 100 women to attend this clinic are the basis for this report. The Menopause Clinic continues today in relatively unchanged form except for the personnel. Our data[3] strongly support our opinions that (1) large numbers of menopausal women suffer from a variety of physical and emotional problems unique to the menopause, (2) menopausal women lack accurate information about the menopause, and (3) the establishment of multidisciplinary menopausal centers is a meaningful and important undertaking.

From the Department of Reproductive Medicine, University of California San Diego.
Presented in part at the Fourth International Congress on the Menopause, Orlando, Florida, October 28–November 2, 1984.
Received for publication July 15, 1986; accepted August 29, 1986.
Reprint requests: S. Hamburger, Department of Reproductive Medicine T-002, University of California, San Diego, School of Medicine, La Jolla, CA 92093.

Subjects and methods

Subjects. Since patients are either self-referred or referred by a physician, it is clear that the data arising from our Menopause Clinic do not necessarily represent a cross section of menopausal women but would be characteristic of such women who seek assistance. We must therefore emphasize that the conclusions we have drawn must be considered as representing our

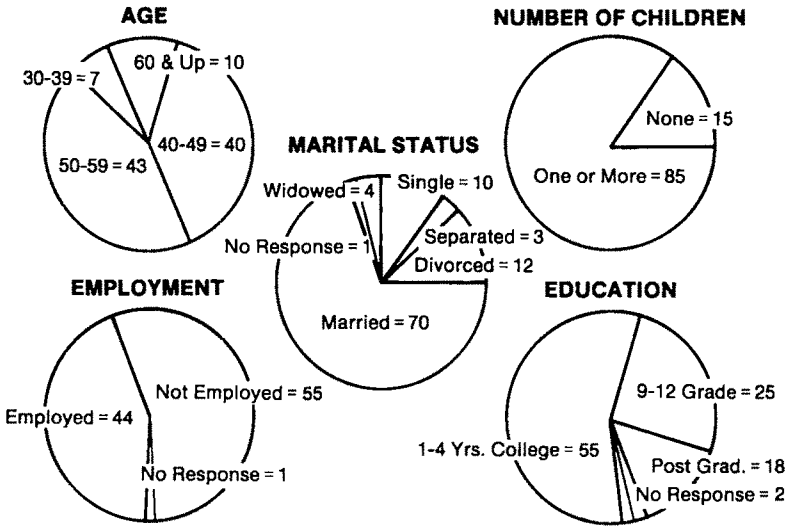


Fig. 1. Demographic distribution of patients seen at the Menopause Clinic from August 1982 to March 1983 by age, marital status, number of children, employment, and education (N = 100).

opinions since no controlled studies were conducted. Young oophorectomized women were neither excluded nor discouraged from attending. The demographic data are summarized in Fig. 1. Most of the first 100 women attending our Clinic from August 1982 to March 1983 were between the ages of 40 and 60 years (range 34 to 71), were currently married, had children, had some education following high school, and about half were employed.

The clinic setting. Participants interact with the health professionals of the clinic in an informal setting after completing a set of detailed questionnaires. Patients summarize their personal needs and, within a permissive and sympathetic atmosphere, are encouraged to share their problems, fears, and frustrations and to take increased responsibility for their own health care by developing a participatory relationship with their physician. Topics explored in depth include symptoms of the menopause, the advantages and disadvantages of hormone replacement therapy, stressing the importance of using progestins concomitantly with estrogens, osteoporosis, surgical menopause, nutrition, exercise, breast self-examination, and mammography. Each patient is given an annotated bibliography citing information about the menopause and related topics, a glossary of terms, a diary in which to keep relevant information on the menopause, dietary and exercise information, and a monthly record sheet on which to record symptoms related to the menopause. Patients are guided and assisted in the decision-making process, that is, whether to discuss hormone replacement therapy with their physician or to seek additional professional consultation from a psychiatrist, career counselor, or nutritionist as the final aspect of the clinic visit. The cinic staff includes a medical anthropologist, a nurse, and a physician with special expertise in reproductive endocrinology for consultation. A telephone number ("hot flash line") is available to patients requiring additional contact with staff. A listing of appropriate referral physicians is maintained for those individuals seeking another physician, but clinic participants are encouraged to share the information acquired with their regular physician. Answers to the questionnaires are made available to the physicians at the patient's request. Follow-up visits are not scheduled routinely but can be arranged on an individual basis if desired by the patient.

Questionnaires. On initial evaluation in the clinic, each patient is asked to complete a symptom checklist, the Zung self-rating depression scale,[4] and a questionnaire that identifies demographic data, family and personal history, height and weight, current medications, and a medical history with chief complaints. Six months later, follow-up questionnaires are mailed to both the patient and, where applicable, the spouse-partner. This survey includes questions about benefits of the menopause clinic visit, actions taken subsequently, and current adjustments with their partner.

Statistics. Data were analyzed with the Statistical Package for Social Sciences (McGraw-Hill). Differences between groups were evaluated with the use of χ^2, contingency coefficient, Kendall's tau, Student's t test, or Pearson's R tests; $p < 0.05$ was considered significant.

Results

Symptoms. The principal reason for attending the clinic varied among patients. Of the 100 participants 79% reported physical symptoms and 63% reported

Table I. Distribution and frequency of physical symptoms

Symptom	%
Hot flashes	80
Muscle/joint pain	72
Headaches	68
Increased weight	65
Lightheadedness	58
Breast sensitivity	56
Increased appetite	50
Dizziness	49
Constipation	45
Numbness and tingling	45
Vaginal discharge	40
Genital pruritus	37

Table II. Distribution and frequency of emotional symptoms

Symptom	%
Irritability	93
Fatigue	91
Tension	91
Nervousness	88
Depression	86
Lack of concentration	82
Short temper	81
Lack of motivation	77
Early awakening	77
Insomnia	77
Loss of memory	75

emotional symptoms as the primary reason for attending the menopause clinic. Further information regarding such complaints was obtained from a symptom checklist. Hot flashes, pain in muscles and joints, headache, and increased weight were the most frequently cited physical symptoms, and irritability, fatigue, tension, nervousness, and depression were the most common emotional-cognitive disturbances (Tables I and II).

As a measure of psychological well-being, Zung self-rating depression scores were obtained for all patients; 35% of the participants had scores in the normal range with the remainder having scores indicative of varying degrees of depression (Table III). Of note is the observation that the Zung depression score was significantly higher ($p < 0.009$ by Student's t test) in those patients (44%) who had had prior extirpative pelvic operations than in those who had not. Thirty percent of all the women had undergone hysterectomy alone and an additional 14% had had hysterectomy and bilateral salpingo-oophorectomy.

Hormone replacement therapy. With regard to estrogen replacement therapy, 64% of women coming to the clinic had received estrogen at some time in the past. However, only about half of these, or 33%, were receiving any estrogen at the time of their clinic appointment. Most of the women using estrogen were receiving it in an unopposed manner without added progestin. More than half of the women with an intact uterus had received estrogen without progestin.

Exercise, weight, and height. Fifty-three percent of the women were obese, as determined from Metropolitan Life Insurance Tables.[5] The majority of women reported they did not exercise regularly. In fact, there was a statistically significant relationship ($p < 0.01$, χ^2) between routine exercise and normal weight; of those with normal weight, 63% exercised routinely, whereas the obese (33%) and grossly obese (28%) reported much less routine exercise. More than half the patients reported no change in height from age of 21; 31% noted a loss of ½ to 1 inch, and 11% reported a loss of 1 to 2 inches.

Sexuality. Only 40% of the women attending the clinic reported that they were satisfied with their sex lives. There was a statistically significant relationship between age and the frequency of intercourse ($p < 0.01$ by χ^2). Of women aged 40 to 49 years, 19% reported that they never or only infrequently had intercourse; this rose to 50% for those aged 50 to 59 years.

When the patients were questioned by mail 6 months later, those who had been satisified with their sex lives remained so. In contrast, dissatisfied patients were still dissatisfied regardless of whether they reported physical improvement with the new use of estrogen replacement. Male partners questioned by mail had quite different responses with regard to satisfaction with their sex lives; 100% of those men with relationships of <20 years reported that they were satisfied while only 50% of those with relationships of more than 20 years' duration reported satisfaction.

Follow-up assessment. Six months after attending the clinic, more than half the women reported that the reason for their appointment was primarily for education and support. Eighty percent of the patients felt that their clinic visit had been useful. Of those who had reported hot flashes, one third stated that their symptoms had become less frequent and/or less intense in the interim, in most cases as a result of the use of estrogen replacement. Almost half of the male partners felt that the menopause had had a negative effect on their relationships; the women were described as "touchy," with difficult mood swings and decreased sexual responsiveness. It should be noted that one third of the male respondents also stated that they themselves were involved in major midlife changes.

Comment

Information that we have accumulated at the Menopause Clinic at the University of California San Diego

Table III. Relationship between Zung self-rating depression score and surgical procedures (N = 95)

Total Zung score (range 30-89)	*Uterus and ovaries intact*		*Hysterectomy alone*		*Hysterectomy and bilateral oophorectomy*	
	n	%	*n*	%	*n*	%
Normal (30-49)	28	54.9	13	43.3	5	35.7
Mild depression (50-59)	15	29.4	7	23.3	6	42.9
Moderate depression (60-69)	5	9.8	5	16.7	2	14.3
Severe depression (>70)	3	5.9	5	16.7	1	7.1
Total	n = 51		n = 30		n = 14	

Medical Center has led us to reassess our approach to dealing with the problems of menopausal women. Many of our misconceptions were corrected by considering the data, although most of the findings were neither surprising nor unexpected.

The fact that 79% of those patients presenting with requests for assistance reported physical symptoms associated with the menopause certainly dispels the myth that "it is all in the head." The numerous objective measurements of hot flashes and their associated physiologic changes confirm the multiple effects of vasomotor instability.[6] The large number of emotional symptoms also reported by our patients strongly suggests the impact of physical complaints on the psyche.

The observation that 33% of the first 100 women presenting to the Menopause Clinic had moderate to severe depression on the basis of the Zung self-rating depression scale indicates the necessity of screening women with menopausal complaints for depression requiring professional care. The strong statistical relationship between depression by this objective scale and the patients who had undergone hysterectomy-oophorectomy suggests that many women undergoing pelvic operation either have not been reconciled to its necessity or were insufficiently prepared for the consequences. Clearly, education and support appear to be warranted both before and after such operations. Despite this observation, however, we would contend that the physical and emotional problems related to estrogen deficiency should be addressed first. Psychiatric treatment is not the first approach to the emotional lability of the menopause.

Classical endocrine teachings have stressed the association between obesity and the peripheral conversion of androgens to estrogens in fat.[7] Such data have led to the widespread conclusion that only thin women will complain of menopausal symptoms. The fact that more than half the women presenting to the clinic were overweight and that fully one fourth were at least 20 pounds overweight surprised us and quickly dispelled this misconception. It is clear that any postmenopausal woman, regardless of weight, may note symptoms related to the menopause. Since so many of our patients are overweight, we emphasize the importance of a calcium-rich, well-balanced diet and a program of regular exercise.[8] Diets should be scrutinized to ensure ingestion of approximately 1 gm/day of elemental calcium[9] in premenopausal women and in women receiving estrogen. For postmenopausal women not receiving estrogen, calcium intake should be increased to 1.5 gm/day. Exercise programs must be tailored to the individual woman. We also encourage regular activity emphasizing the many benefits relative to weight loss, prevention of bone loss, cardiovascular fitness, and mood.

One other common belief has been the notion that menopausal complaints tend to be more common in women suffering from the so-called "empty nest" syndrome, that is, homemakers depressed by the fact that their children have all left home. There was no statistical relationship between severity of depression and the presence or absence of children in the home. There were equal numbers of patients employed outside the home and homemakers. The majority of those employed were professional women who presented because their menopausal symptoms were interfering with their ability to function both outside and within the home. It was the rare patient, employed or unemployed, who expressed sadness or regret that her children had left home.

Not surprisingly, one of the major concerns of the patients revolved around the risks and side effects of hormone replacement therapy: uterine bleeding, weight gain, nausea, edema, breast tenderness, and fear of cancer.[10] Although almost two thirds of our patients had been prescribed estrogens previously, only half of those were still taking estrogen at the time of the clinic visit. Even those taking estrogen were sufficiently concerned about the use of the hormone to attend the clinic. Also of note is the observation that more than half the women taking estrogen who had intact uteri were taking it in an unopposed fashion without added progestin. Almost all of the women taking unopposed estrogen were prescribed this medication by a primary care physician other than a gynecologist. These data

are similar to a report from the Food and Drug Administration.[11] This last survey indicated that during the past 44 years, there appears to have been an increase in the use of progestins and estrogens together. Given the recognized risk of endometrial hyperplasia and carcinoma in women taking estrogen alone,[12, 13] the need for gynecologists to disseminate this information to other physicians is obvious. We believe that educating other physicians as to the benefits and risks of estrogen therapy is a responsibility that gynecologists must undertake and not abdicate.

Another major concern of our patients was the risk of osteoporosis in the postmenopausal years.[14] The only physical recordings that we take at our clinic are measurements of height and weight. We have used height loss as the basis for a discussion of osteoporosis, preventive considerations, and the likelihood of its development in individuals. We have chosen to leave to the patients' individual physicians the decision as to whether objective measurement of bone density is indicated. The risk of osteoporosis has led to intense interest in evaluating bone density in menopausal women. Several reliable noninvasive methods are available for evaluating bone density, and it is generally agreed that estimates of spinal bone density are more accurate as prognostic indicators than are estimates of appendicular (especially radial) bone density. We do not believe that bone density[15] need be estimated in all menopausal women. Rather we encourage measurement of spinal bone density by dual-photon absorptiometry in women at high risk for osteoporosis (that is, those who are white, slender, or receiving glucocorticoids, have a family history of osteoporosis, are cigarette smokers, have heavy caffeine and/or alcohol ingestion, or had early spontaneous or induced menopause) and in those who cannot or choose not to take estrogens in the postmenopausal years. However, because radiation exposure is so low with both dual- and single-photon absorptiometry, we do not feel that either of these procedures is contraindicated in any individual requesting an estimate of bone density. We do not make routine use of quantitative computerized tomographic scanning because of the radiation exposure (tenfold greater than with dual-photon absorptiometry) and the cost. There is probably no need for repeating bone density measurements more frequently than once a year.

In the follow-up questionnaire, the patients reported symptomatic improvement with the use of hormone replacement therapy. However, problems with their sex lives and with sleep generally persisted. It would seem that problems with sexual relationships do not automatically resolve with improvement in physical symptoms. The facts that the male partners often had different perceptions of the quality of the sexual relationship and that many of the men were themselves involved in midlife crises no doubt contribute to this observation. We routinely discuss sexuality in the postmenopausal years and encourage couples with difficulties to seek professional assistance. The difficulties with sleep also seem to have a multifactorial basis and have led us to discuss with our patients the differences between insomnia, early awakening, and intermittent sleep. Many of the patients complain of insomnia but on questioning merely awaken early after a sound sleep.

Experience with our Menopause Clinic has indicated that most women know very little about the menopause, and almost all felt that they had been helped by the clinic when questioned 6 months after the visit. Asked about the benefits of attending the Menopause Clinic, the responses were approximately equally divided among three of several choices: Some women were encouraged to seek medical care; others found relief in the knowledge that many women suffered from similar complaints; still others felt that they had gained helpful new information about the menopause. In any case these data support our belief that multidisciplinary centers for the menopause are needed and are of potential benefit.

Not only have the patients learned from the clinic but those of us health professionals involved with its operation have benefited as well. We believe that there are at least two important implications of our findings to the health care of menopausal women. First, each patient must be an informed partner in making decisions regarding therapy. Patients will no longer adhere to medical advice that has not been adequately explained and with which they therefore do not agree. This seems to be especially true with regard to treatment for the menopause, which is not life-threatening and in which the quality of life is involved. Women prescribed estrogens but not given sufficient information do not take them long. Second, treatment of menopausal problems must be individualized for the specific woman.

The Menopause Clinic described in this paper enables the gathering of longitudinal epidemiologic data at one center. Research on the effects of various treatments can be planned, developed, and evaluated. Screening for osteoporosis can be provided. All involved specialities (endocrinology, radiology, psychosocial, exercise physiology, nutrition, etc.) can be effectively coordinated. In the future, we believe, a well-woman's clinic for the climacteric would be the logical progression from the family planning clinic.

Progress is being made in bringing the subject of menopause "out of the closet" and disseminating research findings. However, optimal care of menopausal women requires the cooperation of a knowledgeable and sensitive physician with an interdisciplinary team

of health care professionals dedicated to the treatment of mature women.

We are grateful to Dr. S. S. C. Yen for his support and suggestions in the formation of the Menopause Clinic under the auspices of the Department of Reproductive Medicine. We are also indebted to the staff of the Menopause Clinic: Jeannette Fonseca, R.N., Lucy Bedard, R.N. (research assistant), Norma MacDaniels, L.V.N., Caroline Frazier, and Karen Gary. We also thank Del Alsobrook for her assistance with the manuscript.

REFERENCES

1. Utian W. Menopause clinics: purpose, function and international comparison. In: Third international congress on the menopause, workshop 15. Lancaster, England, and Boston, Massachusetts: MTP Press, 1981:147-161.
2. Budoff PW. No more hot flashes and other good news. New York: GP Putnam's Sons, 1983.
3. Rebar RW, Anderson E, Hamburger S, Liu J. Characteristics of perimenopausal women seeking assistance [Abstract No. 109]. Orlando: Fourth international congress on the menopause, October 28–November 2. Maturitas 1984;6:167-8.
4. Zung WK. A self-rating depression scale. Arch Gen Psychiatry 1965;12:63.
5. Height and weight tables. New York: Metropolitan Life Insurance Co 1983. [Available from the Health and Safety Education Division, Metropolitan Life Insurance Co., One Madison Ave., New York, NY 10010.]
6. Casper RF, Yen SSC, Wilkes MM. Menopausal flushes: a neuroendocrine link with pulsatile hormone secretion. Science 1979;205:823.
7. Groden JM, Siiteri PK, MacDonald PC. Source of estrogen production in postmenopausal women. J Clin Endocrinol Metab 1973;36:207.
8. Gregory C. Possible influence of physical activity on musculoskeletal symptoms of menopausal and postmenopausal women. J Obstet Gynecol Neonatal Nurs 1982; 22(2):103.
9. Heaney RP, Recker RR, Saville PD. Menopausal changes in calcium balance performance. J Lab Clin Med 1978; 92:953.
10. Hammond CB, Maxson WS. Current status of estrogen therapy for the menopause. Fertil Steril 1982;37:5.
11. Kennedy DL, Baum C, Forbes MB. Noncontraceptive estrogens and progestins: use patterns over time. Obstet Gynecol 1985;65:441.
12. Ziel AK, Finkle WD. Increased risk of endometrial carcinoma among users of conjugated estrogens. N Engl J Med 1975;293:1167.
13. Gambrell RD. Clinical use of progestins in the menopausal patient: dosage and duration. J Reprod Med 1982;27:531.
14. Nachtigall LE, Nachtigall RH, Nachtigall RD, Beckman EM. Estrogen replacement therapy: a 10 year prospective study in the relationship to osteoporosis. Obstet Gynecol 1979;53:277.
15. Genant HK, Cann CE, Ettinger M, et al. Quantitative computed tomography of vertebral spongiosa: a sensitive method of detecting early bone loss after oophorectomy. Ann Intern Med 1982;97:699.

Tocolysis with oral magnesium

Rick W. Martin, M.D., Donald K. Gaddy, M.D., James N. Martin, Jr., M.D., John A. Lucas, M.D., Winfred L. Wiser, M.D., and John C. Morrison, M.D.
Jackson, Mississippi

Seventeen patients in whom uterine activity responded favorably to parenteral magnesium sulfate were given oral magnesium gluconate for continued tocolysis. The mean serum magnesium level before therapy was 1.44 ± 0.22 mg/100 ml, whereas 2 hours after initiation of oral magnesium it was 2.16 ± 0.32 mg/100 ml ($p < 0.05$). One patient had nausea without vomiting or diarrhea. These data suggest that magnesium ingested orally can raise the serum magnesium level significantly. (Am J Obstet Gynecol 1987;156:433-4.)

Key words: Preterm labor, tocolysis, oral magnesium

After uterine activity is abolished with parenteral magnesium sulfate, patients are usually given β-adrenergic receptor agonist drugs orally for continued inhibition of contractions.[1] Magnesium salts are not administered for tocolysis because of gastrointestinal side effects. Because oral therapy with β-sympathomimetics is expensive and often complicated by cardiovascular problems,[2] we undertook a study to observe the effect of oral magnesium gluconate administered for tocolysis in patients with arrested uterine activity.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Mississippi Medical Center.
Supported in part by the Vicksburg Hospital Medical Foundation.
Presented in part at the Thirty-third Annual Meeting of the Society for Gynecologic Investigation, Toronto, Ontario, Canada, March 19-22, 1986.
Received for publication May 20, 1986; revised July 30, 1986; accepted August 22, 1986.
Reprint requests: Rick W. Martin, M.D., Department of Obstetrics and Gynecology, University of Mississippi Medical Center, 2500 North State St., Jackson, MS 39216-4505.

Seventeen patients with documented preterm labor were entered in this study from July 1 through September 30, 1985. On admission, a baseline serum magnesium level was obtained. Each patient was given a bolus and then continual magnesium sulfate intravenously (2 to 3 gm/hr) until uterine activity ceased. All patients in whom effective tocolysis was achieved with intravenous magnesium sulfate were eligible for inclusion in the study. Informed consent was obtained before oral magnesium therapy. All of those eligible agreed to participate. Once tocolysis was effective in reducing uterine activity to less than one contraction every 15 minutes for a period of 4 to 12 hours, the parenteral magnesium was discontinued. After this, 1 gm (two 0.5 gm tablets) of magnesium gluconate was administered to each patient orally every 4 hours. A serum magnesium level was obtained 2 hours after an oral dose of magnesium gluconate between 12 and 24 hours after discontinuation of parenteral magnesium sulfate. Patients were observed for uterine activity and for any evidence of gastrointestinal side effects. Statistical analysis of serum magnesium levels in these patients was performed by the Student paired t test with $p < 0.05$ being considered significant.

The mean serum magnesium level before any therapy was 1.44 ± 0.22 mg/100 ml. Two hours after 1 gm of magnesium gluconate was administered orally, the mean level was 2.16 ± 0.32 mg/100 ml ($p < 0.05$). No patient developed any signs of magnesium toxicity whether the agent was administered parenterally or orally. There was one patient who had nausea but vomiting did not develop and no subject had diarrhea at this dosage of magnesium gluconate. No repeat episodes of preterm labor occurred during this hospitalization.

These data reveal that oral magnesium gluconate can be administered to patients after tocolysis with parenteral magnesium sulfate and that a statistically significant increase in magnesium levels occurred when compared with the pretreatment value. It is possible that the elevated levels might have been due to the previously administered intravenous magnesium sulfate even though 12 to 24 hours had elapsed between discontinuance of the parenteral drug and sample collection. Therefore, we studied five patients in whom labor was arrested with magnesium sulfate as in the above patients but in whom oral β-sympathomimetic agents were given for continued tocolysis. In these five study subjects a level not significantly different from pretreatment values was obtained within 12 to 24 hours after the magnesium sulfate was discontinued (1.57 ± 0.29 mg/100 ml). Thus it would appear that the elevation in magnesium levels noted in our patients does indeed reflect the ingestion of oral magnesium gluconate.

Of special note is that only one patient complained of nausea and she did not have vomiting. In addition, there were no cases of diarrhea, which is another complication that may occur after such therapy. Also at these serum levels there were no cases associated with symptoms of systemic magnesium toxicity. Therefore magnesium gluconate administered in this fashion appears to be safe and relatively free of adverse side effects.

Finally, this drug, while safe, may or may not be an effective oral tocolytic. Reports in the European literature suggest that other forms of oral magnesium (oxide and peroxide) are effective.[3] In our patients, none of the 17 developed recurrent uterine activity or required further parenteral treatment. Nevertheless, since the level of magnesium after oral therapy was much less than the "therapeutic" range (4 to 8 mg/100 ml) found in those treated with parenteral magnesium sulfate for preterm labor or pregnancy-induced hypertension, it remains unclear whether oral treatment with magnesium gluconate will be a clinically effective modality. If it is shown to be an adequate tocolytic, then it will certainly be more cost-effective than β-adrenergic receptor agonist therapy since the cost of ritodrine hydrochloride is approximately $62 per week as compared with the magnesium gluconate expense of $7 for 7 days.

REFERENCES

1. Elliott JP. Magnesium sulfate as a tocolytic agent. Am J Obstet Gynecol 1983;147:277.
2. Thiagarajah S, Harbert GM Jr, Bourgeois FJ. Magnesium sulfate and ritodrine hydrochloride: systemic and uterine hemodynamic effects. Am J Obstet Gynecol 1985;135:666.
3. Rusu O, Lupan C, Battescu V, et al. Magnezial serie in sarcina normala la termen si nasterea permatura. Rolul magneziterapici in combatera nasterii premature. Obstet Ginecol 1966;14:215.

Maternal hypothermia: An unusual complication of magnesium sulfate therapy

John F. Rodis, M.D., Anthony M. Vintzileos, M.D., Winston A. Campbell, M.D., Jeffrey L. Deaton, M.D., and David J. Nochimson, M.D.

Farmington, Connecticut

Reported is a case of maternal hypothermia associated with magnesium sulfate therapy for treatment of preterm labor. Hypothermia was accompanied by fetal and maternal bradycardia. After discontinuation of magnesium sulfate infusion, maternal temperature, pulse, and fetal heart rate gradually returned to normal. No adverse effects were noted on either maternal or fetal outcome. (AM J OBSTET GYNECOL 1987;156:435-6.)

Key words: Hypothermia, magnesium sulfate

Magnesium sulfate is commonly used in the treatment of preeclampsia and preterm labor. Known maternal side effects are respiratory depression, electrocardiogram abnormalities (increased P-R interval, prolonged QRS interval, increased T wave), depression of deep tendon reflexes, and cardiac arrest.[1] To the best of our knowledge this is the first case report of maternal hypothermia secondary to magnesium sulfate therapy.

Case report

B. H., a 30-year-old woman, gravida 7, para 2-1-3-3, was transferred to the University of Connecticut Health Center at 30⅚ weeks' gestation because of preterm labor. Past obstetric history was significant for a preterm vaginal breech delivery, two spontaneous vaginal deliveries at term, a molar pregnancy, and two elective first-trimester abortions.

Physical examination revealed an oral temperature of 99.8° F, pulse 80 bpm, respirations 20/min, and blood pressure 130/70 mm Hg. Careful speculum examination revealed the cervix to be closed and uneffaced with no vaginal bleeding noted. Ultrasound revealed a single viable fetus in a vertex presentation with a complete placenta previa. Amniotic fluid volume appeared adequate. Estimated fetal weight was 1825 gm. Fetal biophysical profile was reassuring. An external fetal monitor revealed uterine contractions every 8 to 10 minutes and a baseline fetal heart rate of 140 to 150 bpm.

The patient received Celestone, 12 mg intramuscularly, to promote fetal pulmonary maturity and a 4 gm intravenous loading dose of magnesium sulfate. A maintenance infusion of magnesium sulfate was initiated at a rate of 2 gm/hr. Persistent contractions necessitated an increase in the infusion to 3 gm/hr, which eliminated all uterine activity. After 2 hours at the new infusion rate the serum magnesium level was 5.2 mg/dl. Maternal vital signs were oral temperature of 97° F, pulse 94 bpm, and blood pressure 120/80 mm Hg.

Twelve hours after admission the fetal heart rate was noted to be 110 bpm. The maternal vital signs were rectal temperature 95.8° F, pulse 64 bpm, blood pressure 90/50 mm Hg, and respirations 18/min. The patient complained of lethargy and diplopia. In spite of a serum magnesium level of only 6.6 mg/dl, the magnesium sulfate infusion was discontinued.

During the next 6 hours the patient's visual and cerebral abnormalities cleared. Maternal temperature slowly increased, and 6 hours after cessation of magnesium sulfate therapy vital signs were temperature 98° F, pulse 80 bpm, and blood pressure 120/60 mm Hg. No uterine contractions or bleeding was noted and baseline fetal heart rate returned to 130 to 140 bpm with normal variability.

Forty-eight hours after admission the patient had an episode of heavy vaginal bleeding, uterine contractions, and fetal tachycardia. A primary cesarean section was performed. A 1790 gm female infant was delivered with Apgar scores of 3 and 8 at 1 and 5 minutes, respectively. Arterial and venous cord pH values were 7.28 and 7.35, respectively. Placental cultures taken were later reported positive for *Listeria monocytogenes.*

Comment

In humans, body temperature is maintained within a narrow range despite extremes in environmental conditions and physical activity. The control of body temperature is a function of cerebral centers located in the hypothalamus. Oral temperatures <96° F are rarely encountered.[2] Rectal temperatures are usually 0.5° to

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, The University of Connecticut Health Center.

Received for publication July 15, 1986; revised August 22, 1986; accepted August 27, 1986.

Reprint requests: John Rodis, M.D., Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, The University of Connecticut Health Center, Farmington, CT 06032.

1.0° F higher than oral values. When there is a strong stimulus for heat production, muscle activity may increase to the point of shivering or even to a generalized rigor.

Instillation of cool intravenous fluids could have caused the observed hypothermia, but this is an unlikely cause in this patient. The fact that the temperature gradually returned to normal after discontinuation of magnesium sulfate, even though the total infusion rate remained constant, implicated the drug rather than the fluids as the source of hypothermia.

Hypothermia may also occur in patients with myxedema or infection. Since the patient was clinically euthyroid before and after the episode of hypothermia and had no history of thyroid disease, these are unlikely causes. Despite the recovery of *Listeria monocytogenes* from the placenta, the prompt resolution of hypothermia after cessation of magnesium sulfate therapy would support that magnesium sulfate rather than infection was the cause of the significant decrease in body temperature in this patient.

In conclusion, it appears that maternal hypothermia may be a complication of intravenous magnesium sulfate administration. One could hypothesize that magnesium sulfate might act centrally at the hypothalamic level or peripherally at the motor end plate to suppress muscle activity, hence causing decreased heat production that results in hypothermia. It is possible that milder degrees of hypothermia occur more commonly in patients receiving magnesium sulfate. However, this may not be brought to the attention of the physician, especially in the setting of severe preeclampsia or premature labor, when the attendant would be most concerned with other vital signs. The effect of magnesium sulfate on maternal temperature regulation appears to be reversible and does not seem to be associated with significant maternal or neonatal morbidity.

REFERENCES

1. Wacker WEC, Pavis AF. Magnesium metabolism. N Engl J Med 1968;278:65.
2. Petersdorf RG. Disturbances of heat regulation. In: Petersdorf RG, Adams RD, Braunwald E, et al. eds. Harrison's principles of internal medicine. 10th ed. New York: McGraw-Hill, 1983:50-7.

Plasma progesterone concentration in women with and without adenocarcinoma of the endometrium

Andrew P. Bond, M.D., Michael J. Diver, M.Sc., and J. Collinson Davis, M.D.
Liverpool, England

In the search for the postulated but elusive "estrogenic bias" in patients developing endometrial cancer, several authors have suggested lack of progesterone as the common denominator. In this study 21 patients at the time of diagnosis of Stage I disease are compared to stringently matched healthy control subjects. No significant difference was found in plasma progesterone concentrations between the two groups. These results, in conjunction with other calculated estrogenic indices based on these patients, provide no evidence of lack of progesterone at the onset of clinical disease. However, the effects of long-term progesterone lack related to chronic anovulation are not excluded by these findings. (Am J Obstet Gynecol 1987;156:437-40.)

Key words: Progesterone, uterine neoplasms, endometrium

Indices of estrogenicity in patients with endometrial cancer have been measured with use of a variety of endocrine parameters. Based on estrogen or androgen production[1-4] and transport,[5, 6] these measurements, when adequately controlled for body mass, tend to reveal little or no "estrogenic bias" in patients developing the disease. Gambrell,[7] along with other authors,[8-9] has presented the case for progesterone lack rather than estrogen overproduction as being the key to this elusive estrogenicity. It was therefore decided to assess the progesterone status of women at the diagnostic phase of Stage I disease and compare it with that of stringently matched control subjects.

Material and methods

Patient sample. Twenty-one patients presenting with FIGO Stage I cancers were matched with controls without endometrial-cancer for the principal epidemiologic parameters associated with the disease (Table I). Adequacy of matching was demonstrated by the detection of no significant difference between the groups for the parameters of age (t, paired = 0.256, $p > 0.5$), years since menopause (t, paired = 0.724, $0.5 > p > 0.1$), bulk index (weight/height^2)[11] (t, paired = 0.622, $p > 0.5$), and parity ($\chi^2 = 4.48$, $p > 0.5$).

Venipuncture was performed before noon and before any diagnostic or therapeutic procedure.

Progesterone assay. A danazol displacement assay was modified to embrace the range 0.080 to 4.90 ng/ml.[12] The following reagents were used: rabbit anti-progesterone α-hemisuccinyl conjugate (a gift from Dr. J. Corrie); danazol in crystalline form (Sterling-Winthrop); iodine 125–progesterone α-glucuronyl-tyramine (Amersham International); sheep antirabbit serum in cellulose matrix (Sac-cel, Wellcome Reagents Ltd.). Assay details are available from us. Definitive quality control experiments produced an intra-assay coefficient of variation of 4.2%. All samples in the study were assayed as a single batch with reference high and low controls.

Statistical analysis. Results were compared with use of Student's one-tailed t test on log values of plasma concentration. As a nonparametric check, the Wilcoxon rank sum test was also employed.

Results

The results are summarized in Table II. They indicate no significant difference in plasma progesterone concentration between the two groups. Plainly, patients F3 and C7 were mistaken concerning their menopausal status. However, even if these suboptimally matched pairs are excluded from the comparison, the difference remains nonsignificant.

Comment

The theory of progesterone lack is based on the cumulative effects of chronic anovulation. To measure progesterone levels at a single point in time, as in this study, is therefore not an appropriate test of the theory, which remains unchallenged by the findings. However, since estrogenic stimulation is more likely to promote than actually initiate mutation of an endometrial cell,[13] it is reasonable to suppose that such an influence would still be present, albeit attenuated, at the time of diagnosis.

From the Departments of Gynaecology and Endocrine Pathology, University of Liverpool.
Received for publication March 20, 1986; revised August 4, 1986; accepted August 22, 1986.
Reprint requests: A. P. Bond, M.D., University Department of Gynaecology, Royal Liverpool Hospital, Liverpool, L7 8XP England.

Table I. Details of patients and control subjects in plasma studies

	Patients with endometrial adenocarcinoma				
Patient No.	*Age*	*Parity*	*Years since menopause*	*Bulk index*	*Presence/absence of significant disease*
A1	42	0 + 0	SM	22.06	Carcinoma of the colon
A2	52	7 + 0	2	25.98	Hypertension, Graves
A3	58	2 + 0	7	21.23	
A4	64	3 + 0	8	30.29	
A5	55	1 + 0	3	26.31	
A6	53	3 + 0	2	26.17	
D5	64	0 + 0	14	25.76	Hypertension, carcinoma of the breast
D6	68	1 + 0	14	24.14	Autoimmune thyroiditis
D8	50	2 + 0	6	23.63	
D11	51	2 + 0	1	34.10	
D16	51	4 + 0	10	20.29	Hypertension
D17	61	1 + 1	6	26.64	
D18	58	3 + 0	8	21.56	
D21	81	4 + 0	31	22.64	Maturity-onset diabetes
D22	52	0 + 0	2	23.18	
D25	62	0 + 0	8	24.11	Hypertension
D31	68	0 + 0	21	21.13	
F2	63	2 + 0	7	32.91	
F3	52	0 + 1	1	33.69	Hypertension
G1	67	3 + 3	27	23.94	
G2	76	2 + 0	23	24.84	

SM = Still menstruating.

Table II. Comparison of plasma progesterone concentration in patients and control subjects

Patient No.	*Plasma progesterone (ng/ml)*	*Log progesterone concentration*	*Control No.*	*Plasma progesterone (ng/ml)*	*Log progesterone concentration*
A1	0.178	−0.751	C1	0.271	−0.567
A2	0.284	−0.547	C2	0.206	−0.685
A3	0.523	−0.282	C3	0.310	−0.510
A4	0.190	−0.721	C4	0.245	−0.661
A5	0.271	−0.567	U1	0.339	−0.470
A6	0.371	−0.431	C5	0.416	−0.381
D5	0.294	−0.532	H1	0.406	−0.391
D6	0.245	−0.611	U2	0.161	−0.792
D8	0.348	−0.458	C6	0.258	−0.588
D11	0.377	−0.423	C7	7.774*	0.891
D16	0.316	−0.500	H2	0.416	−0.381
D17	0.197	−0.706	C8	0.587	−0.231
D18	0.223	−0.653	U3	0.142	−0.848
D21	0.926	−0.033	U4	0.310	−0.510
D22	0.265	−0.578	C9	0.319	−0.496
D25	0.339	−0.470	H3	0.248	−0.605
D31	0.374	−0.427	C10	0.303	−0.518
F2	0.258	−0.588	C11	0.139	−0.858
F3	8.548*	0.932	H4	0.277	−0.557
G1	1.177	0.071	C12	0.219	−0.659
G2	0.394	−0.409	C13	0.232	−0.634
Mean	0.767	−0.413		0.647	−0.495
±SD (n − 1)	1.80	0.37		1.65	0.35

Significance: $t = 0.74$, $0.25 > p > 0.2$—NS (Student's one-tailed t test). $z = 0.969$, $0.5 > p > 0.1$—NS (Wilcoxon rank sum test). If pairs D11/C7 and F3/H4 are excluded, $t = 1.0069$, $0.15 > p > 0.1$—NS.

*Verified on routine laboratory assay.

In the postmenopausal woman the adrenal gland is the sole significant source of production of progesterone, and this serves as a precursor of the adrenocortical steroids. Its relevance to the senescent endometrium is therefore questionable. However, considering the apparent changes in molecular structure of the estrogen receptor in the aging rat endometrium[14] in the presence of retained binding capacity of progesterone recep-

Subject No.	Matched control subjects				
	Age	*Parity*	*Years since menopause*	*Bulk index*	*Presence/absence of significant disease*
C1	42	0 + 1	SM	19.38	Carcinoma of the breast
C2	52	5 + 0	2	25.99	
C3	55	3 + 0	7	24.84	
C4	61	2 + 0	7	25.44	
U1	55	2 + 0	4	25.04	
C5	52	4 + 0	2	27.99	
H1	60	0 + 0	25	23.63	Hypertension
U2	68	2 + 3	22	31.80	
C6	51	3 + 1	4	20.65	
C7	54	2 + 1	1	36.67	
H2	53	5 + 0	12	23.73	
C8	62	1 + 0	8	26.01	
U3	62	4 + 0	12	22.40	
U4	78	5 + 0	25	23.25	Maturity-onset diabetes
C9	55	0 + 0	1	21.49	
H3	65	0 + 0	10	22.23	Hypertension
C10	73	1 + 1	25	21.08	
C11	61	4 + 0	8	32.58	
H4	55	0 + 0	3	21.56	Hypertension
C12	67	9 + 0	15	25.44	
C13	73	5 + 0	23	22.42	

Table III. Comparison of further indices of estrogenicity in patients and controls

Parameter	*Patients (mean ± SD)*	*Controls (mean ± SD)*	*Significance*
% of free progesterone	4.10 ± 1.05	2.87 ± 1.59	$p > 0.1$*
% of free estradiol	2.27 ± 0.65	2.12 ± 0.56	$p > 0.5$*
Plasma estradiol (pmol/l)	31.47 ± 28	31.80 ± 23	$p > 0.5$*

*Not significant.

tors,[15] it may be that a baseline production of progesterone is still necessary to maintain endometrial quiescence.

This is the first comparative study of plasma progesterone concentration in women with and without endometrial cancer to be reported in the literature. Of course, to measure progesterone in plasma is to gain only a rough guide as to the total endocrine "signal" being delivered to the endometrium. In the same group of patients and controls, we have assessed several other parameters[16] (Table III). Measurement of percent of free progesterone, percent of free estradiol, and total plasma estradiol level revealed no significant difference between the groups. When estradiol/progesterone ratios were calculated, the former having been corrected for measured sex hormone–binding globulin capacity and relative metabolic clearance, there were again no significant differences. It would therefore seem that in common with other indices of estrogenicity in endometrial cancer, the theory of progesterone lack does not translate well into hard endocrinologic data.

REFERENCES

1. Judd HL, Lucas WE, Yen SC. Serum 17 beta-estradiol and estrone levels in postmenopausal women with and without endometrial cancer. J Clin Endocrinol Metab 1976;43:272-8.
2. Judd HL, Davidson BJ, Frumar AM, et al. Serum androgens and estrogens in postmenopausal women with and without endometrial cancer. Am J Obstet Gynecol 1980;136:859-66.
3. Schindler AE, Ebert A, Friedrich E. Converstion of androstenedione to estrone by human fat tissue. J Clin Endocrinol Metab 1972;35:622-30.
4. Oettinger M, Sarnberg I, Levitan Z, et al. Hormonal profile of endometrial cancer. Gynecol Obstet Invest 1984; 17:225-35.
5. Nisker JA, Hammond GL, Davidson BJ, et al. Serum sex hormone binding globulin capacity and the percentage of free estradiol in postmenopausal women with and without endometrial carcinoma. Am J Obstet Gynecol 1980;138: 637-42.
6. Davidson BJ, Gambone JC, Lagasse D, et al. Free estradiol in postmenopausal women with and without endometrial cancer. J Clin Endocrinol Metab 1981;52:404-8.
7. Gambrell RD. The role of hormones in the etiology of breast and endometrial cancer. Acta Obstet Gynecol Scand 1979;88:73-81.
8. Greenblatt RB. "Auditorum" cassette tape. Suppl 2. Surbiton, England: Sterling Winthrop, 1979.
9. Greenblatt RB, Gambrell RD, Stoddard LD. The protective role of progesterone in the prevention of endometrial cancer. Pathol Res Pract 1982;174:297-318.
10. Gonzalez ER. Chronic anovulation may increase postmenopausal breast cancer risk [News]. JAMA 1983;249: 445-6.
11. Garrow IS. Weight penalties. Br Med J 1979;2:1171-2.
12. Ratcliffe WA, Corrie JET, Dalzeil AH. Direct ^{125}I-radioligand assays for serum progesterone compared with as-

says involving extraction of serum. Clin Chem 1982;28: 1314-8.
13. Collins J. Oestrogen and endometrial cancer—a reappraisal. J R Soc Med 1981;74:403-6.
14. Hsueh AJW, Erickson FG, Lu KH. Changes in uterine estrogen receptor and morphology in aging female rats. Biol Reprod 1979;21:793-800.
15. Saiduddin S, Zassenhaus HP. Estrous cycles, decidual cell response and uterine and progesterone receptor in Fischer 344 virgin aging female rats. Proc Soc Exp Biol Med 1979;161:119-22.
16. Bond AP [M.D. thesis]. Availability of sex steroids to normal and malignant human endometrium. Wales: University of Wales, 1986.

Predictive value of CA-125 levels in advanced ovarian cancer

Bruce Patsner, M.D., and Thomas G. Day, Jr., M.D.
Louisville, Kentucky

Thirty-eight patients with advanced ovarian cancer were monitored with serial serum CA-125 levels until second-look laparotomy or disease progression. Eleven of 15 patients had normal CA-125 levels but positive findings at second-look operation; two had normal levels despite progression of disease. Normal levels do not reliably predict absence of disease. (AM J OBSTET GYNECOL 1987;156:440-1.)

Key words: Ovarian adenocarcinoma, serum CA-125, second-look laparotomy

Recent efforts in tumor immunobiology have resulted in the development of monoclonal antibodies, one of which, OC-125, has been raised against an antigen (CA-125) that is present on the surface of most but not all invasive ovarian epithelial cancers and can be measured in the serum of the patient. This study reports the experience of the Division of Gynecologic Oncology of the University of Louisville School of Medicine with this assay.

Methods and material

Thirty-eight patients with histologically verified invasive ovarian adenocarcinoma (34 serous or anaplastic, one mucinous, one borderline) underwent cytoreductive operation by the Gynecologic Oncology service at the University of Louisville School of Medicine. PAC chemotherapy (cisplatin, Adriamycin, Cytoxan) was administered monthly for a total of 12 courses or until disease progression was documented. Serum CA-125 levels were determined at each visit and measured in the same laboratory according to previously described standard immunoradiometric techniques.[1] A CA-125 level >35 U/ml measured against a known reference standard defined an elevated value. No knowledge of patient disease status was known by the technicians performing the assay, and results of each test were not known to the authors at the time of examination of the patients.

Second-look laparotomy was defined as a full surgical evaluation in a patient who had completed all twelve courses of chemotherapy and had no evidence of disease on complete physical examination and radiologic evaluation. Only positive washings or biopsy specimens were considered evidence of disease.

Results

Twenty-six patients completed 12 courses of PAC chemotherapy and were eligible for second-look laparotomy. Five patients refused operation and all remain without clinical evidence of disease, with normal CA-125 levels and mean length of follow-up of 12 months. Of the remaining 21 patients, six had negative second-look laparotomy and all six had normal preoperative CA-125 levels. One of these patients developed an elevated CA-125 level 1 month before recurrent disease was detected clinically. Fifteen patients had biopsy-verified disease at second-look laparotomy. Eleven (73%) of these patients had normal CA-125 levels at the time of operation although one had a borderline serous tumor. Eight patients had disease <2 cm, one had microscopic disease, and two had 5 cm abdominopelvic tumors. Of the four patients with elevated CA-125 levels at the time of second-look operation, all had levels in the 40 to 75 U/ml range, and none had tumor larger than 2 cm although several had diffuse intra-abdominal carcinomatosis.

Seven patients had failure of first-line chemotherapy. CA-125 levels in five of these patients never fell to normal ranges after operation despite absence of clinical

From the Division of Gynecologic Oncology, Department Obstetrics and Gynecology, University of Louisville School of Medicine.
Received for publication April 1, 1986; revised July 16, 1986; accepted August 12, 1986.
Reprint requests: Bruce Patsner, M.D., Department of Obstetrics and Gynecology, SUNY at Stony Brook, HSC-T-9, Stony Brook, NY 11794.

disease. Two patients never had elevated CA-125 levels despite massive progression of disease with bone and liver metastases and intra-abdominal carcinomatosis. Five patients are receiving first-line chemotherapy and remain without evidence of disease. CA-125 levels fell to normal in this group of patients within three courses of chemotherapy.

Comment

On the basis of the current literature[2] it appears that an elevated serum CA-125 level is more predictive of disease status than a normal level. The results of this study are in agreement with this: No patient with a negative second-look laparotomy had an elevated CA-125 level, and all patients with an elevated level had disease discovered at operation. Unfortunately, 73% of patients with no clinical evidence of disease and normal CA-125 levels had biopsy-verified persistent disease at second look, and two patients who developed extensive disease while receiving first-line therapy never had an elevated CA-125 level. Thus second-look operation cannot be obviated in patients with negative examinations and normal CA-125 levels if such are to be used in deciding whether chemotherapy is to be discontinued. A rising level may, however, be an indication for surgical intervention to document and debulk tumor even in the presence of a normal clinical examination, in order to maximize the efficacy of second-line therapy.

The presence of normal CA-125 levels in patients with extensive occult disease (5 cm) is disturbing and suggests a possible limitation of use of laparoscopy alone as a second-look procedure.

REFERENCES

1. Bast RC, Feeney ME, Lazarus H. Reactivity of monoclonal antibody with human ovarian carcinoma. Proc Am Assoc Cancer Res 1980;21:207.
2. Niloff JM, Bast RC, Schaetzl EM, et al. Predictive value of CA-125 antigen levels in second-look procedures for ovarian cancer. AM J OBSTET GYNECOL 1985;151:981.

Sonographic determination of fetal weights in diabetic pregnancies

Carol B. Benson, M.D., Peter M. Doubilet, M.D., Ph.D., and Daniel H. Saltzman, M.D.
Boston, Massachusetts

Accurate antenatal diagnosis of the macrosomic infant of a diabetic mother should lead to a decrease in perinatal morbidity. Several formulas for weight estimation based on sonographic measurements are in common use, but none of them has been devised specifically for the infant of a diabetic mother. We assessed the accuracy of conventional formulas at estimating fetal weight in 160 of these infants and found their relative errors (error as a percentage of birth weight) had standard deviations of 12.2% to 13.1% (greater than has been reported in the general population) with no statistically significant difference among formulas. We also devised customized formulas generated from a "training set" of 80 patients and tested on a second set of 80 patients. The best formula yielded a standard deviation of 11.0%. The difference between customized and conventional formulas was not statistically significant. With use of one conventional formula, the likelihood of macrosomia was found to be 77% when the estimated weight was >4000 gm and 86% when the estimated weight was >4500 gm. We conclude that fetal weight prediction is even less accurate in the infant of a diabetic mother than in the general population and that "customized" formulas fare no better than conventional formulas in diabetics. Despite this, estimated weights can offer guidance in predicting fetal macrosomia and therefore aid in obstetric management. (AM J OBSTET GYNECOL 1987;156:441-4.)

Key words: Diabetes, pregnancy, ultrasound

From the Departments of Radiology and Obstetrics and Gynecology, Harvard Medical School, Brigham and Women's Hospital, and the Joslin Clinic.
Received for publication April 4, 1986; revised July 17, 1986; accepted August 19, 1986.
Reprint requests: Carol B. Benson, M.D., Department of Radiology, Brigham and Women's Hospital, 75 Francis St., Boston, Massachusetts 02115.

Accurate antenatal diagnosis of the macrosomic (birth weight >4000 gm) infant of a diabetic mother should lead to a decrease in perinatal morbidity. The identification of these fetuses at high risk for traumatic birth injuries would allow for an elective cesarean section as the most appropriate and prudent route of de-

livery.[1] While several formulas for weight estimation based on sonographic measurements have been proposed,[2-5] none of those in common use have been devised specifically for fetuses of diabetic mothers. Since the body habitus of the infant of a diabetic mother is known to differ from the nondiabetic population, we undertook a study to assess the accuracy of conventional weight formulas in fetuses of diabetic mothers and to determine whether formulas specifically designed for diabetic mothers outperform conventional formulas in such fetuses. In addition to examination of the accuracy in prediction of actual fetal weight, a further goal was to determine the ability of current formulas to diagnose or exclude macrosomia.

Material and methods

Case material. We evaluated the sonographic examinations and obtained birth weights of 160 fetuses of diabetic mothers scanned within 1 week of delivery at our institution between January 1983 and February 1985. Specifically excluded from the study were twins and fetuses with congenital anomalies at birth.

All patients studied were insulin-dependent diabetic women before pregnancy or were gestational diabetic women diagnosed by abnormal glucose tolerance testing during pregnancy.[6]

Data collected. The sonographic examinations were performed with use of real-time sector scanners. Measurements collected on all fetuses included biparietal diameter (BPD), femur length (FL), and transverse and anteroposterior abdominal diameters (AD_1 and AD_2). Occipitofrontal diameter (OFD) was obtained whenever possible. The abdominal circumference (AC) was calculated by the formula

$$AC = (AD_1 + AD_2) \times 1.57$$

When the OFD was available, the corrected BPD[7] was calculated and used in place of the BPD in all formulas. Review of all ultrasound studies was performed without knowledge of neonatal birth weight.

All fetuses were weighed at birth. To account for fetal weight gain between the time of sonographic measurements and the date of birth, the actual fetal weight at the time of the examination was assumed to be the birth weight minus 30 gm for each elapsed day. (Thirty grams is the approximate daily weight gain in the third trimester.[8])

Evaluation of current formulas. We evaluated formulas of Warsof et al.,[2] Shepard et al.,[3] and Hadlock et al.[4] (We refer to the formula labeled E2 in Shepard et al.[3] as the "Shepard formula." The "Hadlock formula" used contains the variables AC × FL, BPD^2, AC, and FL.) We determined the error made by each formula on each of our 160 cases by taking the difference between the weight predicted by the formula and the actual weight. The relative error, the error expressed as a percent of the actual weight, was also calculated. The mean and standard deviation of the errors and relative errors were computed for each formula. The F test was used to compare formulas. We evaluated these current formulas:*

Warsof et al.[2]:

$$\log_{10}(FW) = -1.599 + 0.144(BPD) + 0.032(AC) - 0.111(BPD^2 \times AC)/1000$$

Shepard et al.[3]:

$$\log_{10}(FW) = -1.7492 + 0.166(BPD) + 0.046(AC) - 2.646(AC \times BPD)/1000$$

Hadlock et al.[4]*:

$$\log_{10}(FW) = -1.5213 - 0.003343(AC \times FL) + 0.001837(BPD)^2 + 0.0458(AC) + 0.158(FL)$$

where FW = Birth weight (kg), BPD = biparietal diameter (cm), AC = abdominal circumference (cm), and FL = femur length (cm).

Generation and assessment of new formulas. We divided our diabetic population into two equal groups of 80 patients each. The first group ("training set") was used to generate new weight-prediction formulas and the second group ("test set") to evaluate these formulas.

Formulas were generated from the training set of 80 cases by means of computer-assisted multiple regression analysis. For each formula, the dependent variable was $\log_{10}(FW)$ or FW, and a combination of fetal measurements was used as the independent variables. In particular, we developed a "Warsof-variant" formula by using the terms BPD, AC, and $BPD^2 \times AC$—the same terms used in the Warsof formula—as the independent variables. "Shepard-" and "Hadlock-variant" formulas were generated in a similar fashion. Four other formulas, with use of different combinations of measurements and independent variables, were also generated.

These formulas were then assessed on the test set by use of the same methods as were described in the previous section for evaluating current formulas.

Results

The performance characteristics of current formulas in our set of 160 diabetic mothers are presented in Table I. The Shepard and Hadlock formulas had very low systematic errors, in that their mean errors and mean relative errors were small. The standard deviation of the relative error, a measure of a formula's precision at predicting weight, was slightly lower with use of Hadlock et al.'s formula (±12.2%) than that of Warsof et al. (±12.6%) or Shepard et al. (±13.1%). The difference, however, was not statistically significant ($p > 0.05$).

*The constant term (−1.5213) differs from that used in Hadlock et al.[4] (1.4787) because we are expressing weights in kilograms instead of grams.

Table I. Performance characteristics of current formulas (mean ± SD)

Formulas	*Error (%)*		*Error (gm)*	
Warsof et al.	5.9 ± 12.6	*	242 ± 441	*
Shepard et al.	1.6 ± 13.1		102 ± 441	
Hadlock et al.	2.6 ± 12.2		124 ± 427	

*No difference between formulas (F test, $p > 0.05$).

Table II. Performance characteristics of "customized formulas" (mean ± SD)

Formula No.	*Error (%)*		*Error (gm)*	
I	−1.1 ± 12.2*	*	15 ± 409*	*
II	−0.9 ± 12.1		19 ± 409	
III	−0.9 ± 11.0		16 ± 394	
IV	−1.8 ± 11.5		−17 ± 396	
V	−0.9 ± 12.0		27 ± 406	
VI	−5.2 ± 12.6		−128 ± 432	
VII	−2.4 ± 13		−13 ± 415	

*No difference between formulas (F test, $p > 0.05$).

The formulas generated from the first 80 patients (the "training set") are as follows (their performance characteristics, when applied to the "test set," are presented in Table II):

I. (Warsof et al.'s variant)
$$\log_{10}(FW) = -2.579 + 0.267(BPD) + 0.048(AC) - 0.000340(BPD)^2(AC)$$

II. (Shepard et al.'s variant)
$$\log_{10}(FW) = -2.9944 + 0.313(BPD) + 0.0865(AC) - 0.00734(AC)(BPD)$$

III. (Hadlock et al.'s variant)
$$\log_{10}(FW) = -2.080 - 0.00638(AC)(FL) + 0.00265(BPD)^2 + 0.0623(AC) + 0.255(FL)$$

IV. $$FW = -3.457 + 0.0725(FL) + 0.122(AC) + 0.00333(FL)(AC) + 0.0168(BPD)^2$$

V. $$\log_{10}(FW) = -3.509 + 0.618(BPD) + 0.0262(AC) + 0.0645(FL) - 0.0323(BPD)^2 - 0.00109(FL)(AC)$$

VI. $$\log_{10}(FW) = -3.383 + 1.24\log_{10}(BPD) + 1.45\log_{10}(AC) + 0.607\log_{10}(FL)$$

VII. $$FW = 0.0544 - 0.00141(BPD)^3 + 0.00271(FL)(AD)^2$$

The variant formulas of Warsof et al., Shepard et al., and Hadlock et al. had smaller systematic errors and greater precision than their parent formulas, but the differences were minor and not statistically significant. As with current formulas, the variant formula of Hadlock et al. had the greatest precision (that is, the lowest standard deviation of 11.0%), besting the variants of Warsof et al. and Shepard et al. as well as all other formulas generated. None of the differences among formulas, however, was statistically significant ($p > 0.05$).

Table III. Fetal weight estimate versus birth weight

*Estimated fetal weight (gm)**	*Actual weight*			
	<4000 gm		*>4000 gm*	
	n	%	*n*	%
<4000	112	84	22	16
>4000	6	23	20	77

*By the Hadlock formula.

Table IV. Actual weight as a function of prenatal weight estimate in infants of diabetic mothers

*Estimated fetal weight (gm)**	*No. of fetuses*	*% Macrosomic*
<3500	96	7
3500-3999	39	39
4000-4499	19	74
>4500	7	86

*By the Hadlock formula.

We grouped cases according to fetal weight estimate by the Hadlock formula in order to determine the ability to diagnose or rule out macrosomia in the infant of a diabetic mother. The results are listed in Table III. A fetus with a weight estimate <4000 gm has a 16% chance of being macrosomic, whereas a fetus with an estimated weight >4000 gm is likely (77%) to be macrosomic. A further breakdown of our groups for narrower weight ranges is presented in Table IV.

Comment

The macrosomic infant of a diabetic mother is at increased risk for birth trauma secondary to shoulder dystocia or failed forceps delivery, among other problems. The incidence of shoulder dystocia in the general population is 0.5%. This increases to 31% in the macrosomic infant of a diabetic mother.[9] Accurate antenatal fetal weight prediction could therefore lead to a considerable reduction in perinatal morbidity in this group.

Weight prediction has been shown to be of limited accuracy in the general population, and our study has demonstrated even less accuracy in the diabetic population. Specifically, in the general population, conventional formulas have been shown to have broad 95% confidence ranges of 15% to 22%.[4] That is, in 5% of cases the estimated weight will be in error by approximately 20% or more. Our results demonstrate that the conventional formulas have even wider 95% confidence

ranges of 24% to 26% in infants of diabetic mothers (2 SD, based on Table I). Although differences among formulas were slight (and not statistically significant), the Hadlock formula involving BPD, AC, and FL showed a tendency to predict weight more accurately than the Warsof and Shepard formulas. "Customized" formulas, generated with use of a group of diabetic fetuses and evaluated on a second "test set," performed no better than the conventional formulas.

Previous studies have addressed the accuracy of sonographic prediction of fetal macrosomia in diabetic pregnancies.[10-14] The definition of macrosomia has not been standardized, with some authors using a relative weight criterion (≥90th percentile)[11, 12, 14] and others an absolute weight criterion (≥4000 gm).[10, 13] Elliott et al.[10] reported a sensitivity of 87% but a predictive value of only 61% in identifying fetuses with birth weights >4000 grams. Bracero et al.[11] showed a 79% sensitivity and 80% specificity in using abdominal diameter/femur length ratios to identify the large for gestational age (≥90th percentile) infants of diabetic mothers. When we examined the use of a similar ratio, the FL/AC ratio, we found a positive predictive value of only 36% to 43%, suggesting that it has limited clinical utility.[13]

Clinical management decisions regarding route of delivery should be based on many factors. One of these is the risk of birth trauma. The incidence of shoulder dystocia in macrosomic (≥4000 gm) infants of diabetic mothers has previously been shown to be significantly increased.[9] Our results demonstrate a high likelihood (77%) of macrosomia when the estimated weight is at least 4000 gm. These findings taken together, while not conclusive, suggest that cesarean section is the most prudent route of delivery when the estimated fetal weight is ≥4000 gm.

We conclude that: (1) current formulas are even less accurate at predicting actual fetal weight in the diabetic mother than in the general population; (2) they do, however, provide considerable guidance at diagnosing and excluding macrosomia and therefore aid in selecting the most appropriate mode of delivery; (3) there is no evidence to support the use of "customized" weight formulas over conventional formulas in diabetic women; (4) pending further study, the Hadlock formula tested here appears to be the method of choice for weight prediction in diabetic women.

REFERENCE

1. Gabbe SG, Mestman JH, Freeman RK, et al. Management and outcome of class A diabetes mellitus. Am J Obstet Gynecol 1977;127:465.
2. Warsof SL, Gohari P, Berkowitz RL, Hobbins JC. The estimation of fetal weight by computer-assisted analysis. Am J Obstet Gynecol 1977;128:881.
3. Shepard MJ, Richards VA, Berkowitz RL, Warsof SL, Hobbins JC. An evaluation of two equations for predicting fetal weight by ultrasound. Am J Obstet Gynecol 1982;142:47.
4. Hadlock FP, Harrist RB, Carpenter RJ, Deter RL, Park SK. Sonographic estimation of fetal weight. Radiology 1984;150:535.
5. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body, and femur measurements: a prospective study. Am J Obstet Gynecol 1985;151:333.
6. O'Sullivan JB, Mahan CM. Criteria for oral glucose tolerance test in pregnancy. Diabetes 1964;13:278.
7. Doubilet PM, Greenes RA. Improved prediction of gestational age from fetal head measurements. AJR 1984; 142:797.
8. Brenner WE, Edelman DA, Hendricks CH. A standard of fetal growth for the United States of America. Am J Obstet Gynecol 1976;126:555.
9. Acker DB, Sachs BP, Friedman EA. Risk factors for shoulder dystocia. Obstet Gynecol 1985;66:762.
10. Elliott JP, Garite TJ, Freeman RK, et al. Ultrasonic prediction of fetal macrosomia in diabetic patients. Obstet Gynecol 1982;60:159.
11. Bracero LA, Baxi LV, Rey HR, et al. Use of ultrasound in antenatal diagnosis of large for gestational age infants in diabetic gravid patients. Am J Obstet Gynecol 1985; 152:43.
12. Tamura RK, Sabbagha RE, Dooley SL, et al. Real-time ultrasound estimations of weight in fetuses of diabetic gravid women. Am J Obstet Gynecol 1985;153:57.
13. Benson CB, Doubilet PM, Saltzman DH, et al. Femur length/abdominal circumference ratio: poor predictor of macrosomic fetuses in diabetic mothers. J Ultrasound Med 1986;5:141.
14. Tamura RK, Sabbagha RE, Depp R, et al. Diabetic macrosomia: accuracy of third trimester ultrasound. Obstet Gynecol 1986;67:828.

Ureteral obstruction as a complication of the Burch colposuspension procedure: Case report

Gerald B. Applegate, M.D., Katherine M. Bass, M.D., and Carolyn J. Kubik, M.D.
Pittsburgh, Pennsylvania

Major complications of the Burch procedure for stress urinary incontinence are rare. Inadvertent kinking of the ureter during this procedure has been described only once previously in the literature. We present a second such case, hoping to draw attention to this rare but significant complication. (AM J OBSTET GYNECOL 1987;156:445.)

Key words: Stress incontinence, Burch procedure, ureteral obstruction

Suspension of the lateral portion of the upper vagina to Cooper's ligament has become an established technique to correct urinary stress incontinence. First described and performed by Burch[1] in 1961, the procedure has few major complications. Intraoperative damage to the ureter with subsequent obstruction has been described only once previously in the literature but can have serious consequences for the patient, and care must be taken to avoid such an occurrence.

Case report

A 46-year-old, gravida 9, para 8, abortions 1, black woman presented to the outpatient clinic at Magee-Womens Hospital because of involuntary loss of urine for the previous 5 years. She had had total abdominal hysterectomy and right salpingo-oophorectomy for benign disease 10 years before presentation. Pelvic examination revealed no cystocele or urethrocele and preoperative cystometric parameters were within normal limits. The patient was admitted and underwent a Burch colposuspension procedure. Three ligatures of No. 0 Vicryl suture were used bilaterally to suspend the vagina to Cooper's ligament under moderate tension, with approximately 1 cm of free space allowed between the ligament and the vagina. The space of Retzius was drained through a separate stab wound. Blood loss at the time of operation was minimal.

On the third postoperative day, the patient developed a fever to 38.4° C and left flank pain and maintained a low-grade fever during the next several days. Full workup including blood and urine cultures revealed no source of infection. An intravenous pyelogram showed left ureteral obstruction approximately 1 cm above the ureterovesical orifice. Eight days after the original procedure, the patient was taken back to the operating room where she underwent cystoscopy with attempted ureteral catheterization without success. At reexploration, cystotomy was performed and the left ureter was found to be kinked and deviated laterally by the vaginal sutures. These were removed bilaterally and both ureters were catheterized, the free ends being brought down through the urethra. An intraoperative retrograde pyelogram demonstrated correct catheter placement with no obstruction. The bladder was drained suprapubically. The ureteral catheters were removed on the third postoperative day and a repeat intravenous pyelogram just before discharge showed only limited narrowing of the distal left ureter with no obstruction. Three months after operation, the patient denied symptoms of urinary stress incontinence, and her only complaint was of mild suprapubic discomfort.

Comment

The problem of urinary stress incontinence in the woman has been extensively addressed in the literature. In properly selected patients, the Burch retropubic urethrovesical suspension procedure offers a better than 85% long-range cure rate for genuine urinary stress incontinence. Minor complications include bladder trauma and venous bleeding while major complications are infrequent. Only one previous case of ureteral obstruction during a Burch procedure has been reported,[2] also in a patient who had undergone previous abdominal hysterectomy. As in our patient, fibrosis and scarring of the paravesical tissue resulted in kinking of the ureter during colposuspension, with subsequent obstructive uropathy. Early recognition and correction of the problem prevented permanent kidney damage in both patients. This rare complication must be kept in mind when one is attempting Burch colposuspension in patients who have undergone previous pelvic operation.

From the Department of Obstetrics and Gynecology, University of Pittsburgh School of Medicine and Magee-Womens Hospital.
Received for publication July 22, 1986; accepted August 11, 1986.
Reprints not available.

REFERENCES

1. Burch JC. Urethrovaginal fixation to Cooper's ligament for correction of stress urinary incontinence, cystocele, and prolapse. AM J OBSTET GYNECOL 1961;81:281.
2. Marik TG. Kinked ureter with unilateral obstructive uropathy complicating Burch colposuspension. J Urol 1983; 130:135.

Vaginal Gram stain as an immediate detector of group B streptococci in selected obstetric patients

Steven M. Feld, M.D., and John T. Harrigan, M.D.
New Brunswick, New Jersey

During a 19-month period selective vaginal Gram stain and culturing were performed prospectively in all women admitted to the delivery room at risk for low birth weight delivery secondary to preterm premature rupture of the membranes or for prolonged membrane rupture seconcary to term premature rupture of the membranes. Gram stain was performed and read immediately on admission. Cultures were also obtained simultaneously and sent for microbiologic evaluation. All women with Gram stains positive for gram-positive cocci were treated with intravenous ampicillin in the event of labor, unless vaginal culture results were already known and were negative for group B β-hemolytic streptococcus. A total of 72 women had samples taken, and 30 (41.7%) were positive for gram-positive cocci. Nine (30%) of these were subsequently positive for group B β-hemolytic streptococci by culture. There were no cultures positive for group B β-hemolytic streptococci in the Gram stain–negative group. Gram stain sensitivity was 100% for predicting the presence of group B β-hemolytic streptococci in the study population, and specificity was 66.7%. Selective vaginal Gram stain provides an effective and rapid screen for identifying the presence of group B β-hemolytic streptococci and allows for immediate institution of appropriate antibiotic therapy in the event of onset of labor before the availability of culture results. (Am J Obstet Gynecol 1987;156:446-8.)

Key words: Group B streptococci, Gram stain, gram-positive cocci, immediate detection

The two most common bacterial pathogens in the neonatal period are group B β-hemolytic streptococci and *Escherichia coli*, accounting for 70% of all systemic neonatal bacterial diseases.[1] More significant is the ascension of group B β-hemolytic streptococci as a major cause of life-threatening neonatal infection in the United States.[2, 3] An estimated 12,000 to 15,000 newborn infants per year will develop early onset disease, and mortality is at least 50%.[4]

Prevention of this disease in the neonate is a formidable task, as asymptomatic maternal vaginal colonization is common, ranging from 5% to 30% of pregnant women.[1, 5, 6] Vertical transmission at birth is high, occurring in 75% of exposed newborn infants.[7] Fortunately, the attack rate for group B β-hemolytic streptococci is much lower with 1% of colonized infants becoming septic, giving an incidence of 0.6 to 4 per 1000 live births.[5]

Studies attempting to identify and treat antepartum mothers colonized with group B β-hemolytic streptococci have been ineffective in reducing maternal colonization at delivery.[8, 9] Intrapartum screening is more effective in predicting pregnancies that result in neonatal infection,[10] and more recently intravenous ampicillin given intrapartum to pregnant women colonized with group B β-hemolytic streptococci has prevented transmission to the newborn infant.[11, 12]

Certain neonates are at greater risk for developing disease relating to early onset of group B β-hemolytic streptococci, most notably those of low birth weight and those born after prolonged membrane rupture.[2, 3, 7, 13] The attack rate for low birth weight infants is 7.3 times that for term infants,[3] and the mortality rate is at least doubled.[5] Such newborn infants may benefit from intrapartum ampicillin before birth.[13, 14]

Obstetric conditions on occasion may require rapid identification techniques for the presence of maternal vaginal group B β-hemolytic streptococci in order to make informed decisions about initiating intravenous ampicillin before delivery. At present 18 hours or more is required for reliable identification of group B β-hemolytic streptococci,[14, 15] often leading to unnecessary antibiotic therapy. This study examines the effectiveness of the vaginal Gram stain as a rapid and accurate detector of group B β-hemolytic streptococci.

Material and methods

The study population comprised all pregnant women admitted to the delivery room in St. Peter's Medical Center at risk for low birth weight delivery secondary to preterm premature rupture of the membranes or for prolonged membrane rupture secondary to term

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Medicine and Dentistry of New Jersey–Rutgers Medical School.
Received for publication June 20, 1986; revised August 29, 1986; accepted August 31, 1986.
Reprint requests: Steven M. Feld, M.D., Department of Obstetrics and Gynecology, Jersey Shore Medical Center, 1945 Corlies Ave., Neptune, NJ 07753.

Table I. Types of gram-positive cocci isolated

Type	*No.*	*%*
Group B streptococci	9	30
Staphylococcus epidermidis	9	30
Staphylococcus aureus	3	10
Group D streptococci	3	10
α-Hemolytic streptococci	2	6.7
Staphylococcus epidermidis/α-hemolytic streptococci	3	10
Group D α-hemolytic streptococci	1	3.3

Table II. Gram stain and group B culture results

	Group B streptococci positive	*Group B streptococci negative*	*Total*
Gram-positive cocci positive	9	21	30
Gram-positive cocci negative	0	42	42
Total	9	63	72

premature rupture of the membranes. From November 1981 through June 1983, vaginal cultures and Gram stain were obtained simultaneously from all women who fulfilled the above requirements. Preterm pregnancies included all those that had not completed 37 weeks' gestation. Premature rupture of the membranes was defined as rupture of the membranes preceding the onset of labor by any amount of time.

Sterile speculum examination was performed immediately on admission to the delivery room. Two culture swabs were obtained from the outer one third of the vagina. In addition, a third swab was used to streak a slide, and this specimen was allowed to air dry. All specimens were then immediately transported for microbiologic examination. One specimen was inoculated directly into selective Todd-Hewitt broth medium (5% sheep red blood cells, 8 μg/ml of gentamicin sulfate, and 15 μg/ml of nalidixic acid). At 24 hours inoculates were subcultured onto blood agar. Confirmation of group B β-hemolytic streptococci was obtained with the CAMP test. The second swab was treated anaerobically.

The air-dried smear was used to prepare a Gram stain immediately in the following manner by one of three fellows in maternal-fetal medicine. The smear was heat-fixed and stained with Gentian violet for 1 minute. It was then washed in tap water, and Gram's iodine solution was applied for 1 minute. The smear was again washed in tap water and decolorized in 95% ethyl alcohol for 30 seconds. Once again it was washed in tap water and then counterstained for 20 seconds with safranin. As soon as the Gram stain was completed, it was examined microscopically. Any presence of gram-positive cocci in clumps, pairs, or chains or in an isolated instance was considered positive for group B β-hemolytic streptococci for the purposes of this study until culture results were available.

After sterile speculum examination, no further examinations were done in the study population unless labor occurred. Those women with preterm premature rupture of the membranes were managed conservatively. At the end of 1 week's hospitalization, if no signs of labor or chorioamnionitis existed, they were discharged. Those women with term premature rupture of the membranes were observed for 12 hours, and if labor had not ensued by then, intravenous oxytocin induction of labor was begun.

In the event of labor or induction of labor, if group B β-hemolytic streptococci was present by culture, 1 gm of ampicillin was given intravenously every 4 hours until delivery. If culture results were not yet available, decision for treatment was based solely on Gram stain results.

Once culture results were available, Gram stain and culture were matched for each woman in the study population. Degree of correlation between the two was reviewed.

Results

Vaginal Gram stain and culture were obtained in 72 women during the study period. A total of 61 (84.7%) were admitted for preterm premature rupture of the membranes, while 11 (15.3%) were admitted for term premature rupture of the membranes. A total of nine women had positive vaginal cultures for group B β-hemolytic streptococci, yielding a 12.5% colonization rate. This rate is consistent with the range of colonization reported in other populations.[1, 5, 6] Colonization rate in the preterm premature rupture of the membranes population was 11.5% (seven of 61), while the rate with term premature rupture of the membranes was slightly higher, at 18.2% (two of 11). Again both rates fall within the reported range.

Thirty of 72 Gram stains were positive for gram-positive cocci, giving an incidence of 41.7%. Of these 30, nine (30%) were subsequently positive for group B β-hemolytic streptococci by vaginal culture. Table I lists the various organisms isolated by culture in the Gram stain positive for gram-positive cocci population, with their respective percentages of total. Group B β-hemolytic streptococci was the largest subpopulation. Table II shows the relationship between Gram stain findings and group B β-hemolytic streptococci vaginal culture results. There were no vaginal cultures positive for group B β-hemolytic streptococci in the Gram stain negative for gram-positive cocci group.

All nine women with group B β-hemolytic streptococci by vaginal culture were accurately identified on admission to the delivery room by the Gram stain being

positive for gram-positive cocci. No woman subsequently had a positive culture for group B β-hemolytic streptococci if the initial Gram stain had been negative for gram-positive cocci. Gram stain sensitivity for predicting the presence of group B β-hemolytic streptococci vaginally was 100% for the study population. There were no false negative results. Gram stain specificity was 66.7%.

Comment

Early onset group B β-hemolytic streptococci infection can be life-threatening to the newborn infant. The disease course is often rapid and all too frequently leads to a fatal outcome. It is imperative that those neonates at risk be identified intrapartum so that mothers-to-be can be treated with antibiotics to minimize exposure during fetal passage through the birth canal, as suggested by prior studies.[11, 12, 14] This urgency must be balanced by the increased cost and risks that occur with widescale antibiotic therapy, since incidence of maternal carriage for group B β-hemolytic streptococci can be as high as 30%.[2, 14]

A population at high risk for developing early onset disease has been identified in several studies.[2, 3, 7, 10] Screening of those women admitted at risk for delivering low birth weight newborn infants or those born after prolonged membrane rupture would narrow the target population and eliminate the need for universal antepartum screening.

Even with such an approach, at least 18 hours is required from time of vaginal culture for accurate identification of maternal carriers of group B β-hemolytic streptococci.[14, 15] Many obstetric situations may occur that lead to delivery or active labor before group B β-hemolytic streptococci status is known. In preterm pregnancies with premature rupture of the membranes, more than 50% are in active labor within 24 hours, while 70% of pregnancies at term are in labor within 12 hours.[5]

The clinician is thus faced with the choice of treating such patients with antibiotics unnecessarily (if group B β-hemolytic streptococci cultures are subsequently negative) or not treating and exposing the neonate to potential risk (if group B β-hemolytic streptococci cultures are positive). Minkoff and Mead[14] have recently addressed this issue and suggest treating some patients unnecessarily to prevent intrapartum transmission in a population at high risk for early onset disease as described above. This approach is suggested until a simple, rapid, and accurate diagnostic test for group B β-hemolytic streptococci becomes available, thus eliminating the need for unnecessary antibiotics.

The vaginal Gram stain fulfills these requirements to a large extent. It is a simple and rapid test, as well as an inexpensive one, and has been used in the diagnosis of neonatal group B β-hemolytic streptococci disease with preparation of a smear of gastric aspirate.[6] The purpose of this study was to assess the accuracy of the Gram stain in diagnosing group B β-hemolytic streptococci. Of most importance would be its sensitivity, so that few maternal carriers of group B β-hemolytic streptococci are missed in screening a high-risk population.

A sensitivity of 100% in this small study population is promising. Further analyses in larger populations are needed to confirm these findings. If the sensitivity remains good, vaginal Gram stain will provide an easy and cost-effective screen for group B β-hemolytic streptococci.

REFERENCES

1. Avery ME, Taeusch HW. Schaffer's diseases of the newborn. Philadelphia: WB Saunders, 1984:730-1.
2. Pasnick M, Mead PB, Philip AGS. Selective maternal culturing to identify group B streptococcal infection. AM J OBSTET GYNECOL 1980;138:480-4.
3. Boyer KM, Gadzala CA, Burd LI, Fisher DE, Paton JB, Gotoff SP. Selective intrapartum chemoprophylaxis of neonatal group B streptococcal early-onset disease. I. Epidemiologic rationale. J Infect Dis 1983;148:795-801.
4. Baker CJ. Summary of the workshop on perinatal infections due to group B streptococcus. J Infect Dis 1977; 136:137-52.
5. Gibbs RS, Sweet RL. Maternal and fetal infections. In: Creasy RK, Resnik R, eds. Maternal-fetal medicine—principles and practice. Philadelphia: WB Saunders, 1984; 607-13,632-4.
6. Schwarz RH. Perinatal infections. In: Bolognese RJ, Schwarz RH, Schneider J, ed. Perinatal medicine—management of the high risk fetus and neonate. Baltimore: Williams & Wilkins, 1982:313-35.
7. Baker CJ, Barrett FF. Transmission of group B streptococci among parturient women and their neonates. J Pediatr 1973;83:919-25.
8. Hall RT, Barnes W, Krishnan L, et al. Antibiotic treatment of parturient women colonized with group B streptococci. AM J OBSTET GYNECOL 1976;124:630-4.
9. Gardner SE, Yow MD, Leeds LJ, Thompson PK, Mason EO, Clark DJ. Failure of penicillin to eradicate group B streptococcal colonization in the pregnant woman. AM J OBSTET GYNECOL 1979;135:1062-5.
10. Iams JD, O'Shaughnessy RO. Antepartum versus intrapartum selective screening for maternal group B streptococcal colonization. AM J OBSTET GYNECOL 1982;143: 153-6.
11. Yow MD, Mason EO, Leeds LJ, Thompson PK, Clark DJ, Gardner SE. Ampicillin prevents intrapartum transmission of group B streptococcus. JAMA 1979;241:1245-7.
12. Boyer KM, Gadzala CA, Kelly PD, Gotoff SP. Selective intrapartum chemoprophylaxis of neonatal group B streptococcal early-onset disease. III. Interruption of mother-to-infant transmission. J Infect Dis 1983;148: 810-6.
13. Mead PB. Management of the patient with premature rupture of the membranes. Clin Perinatol 1980;7:243-55.
14. Minkoff H, Mead P. An obstetric approach to the prevention of early-onset group B β-hemolytic streptococcal sepsis. AM J OBSTET GYNECOL 1986;154:973-7.
15. Boyer KM, Gadzala CA, Kelly PD, Burd LI, Gotoff SP. Rapid identification of maternal colonization with group B streptococci by use of fluorescent antibody. J Clin Microbiol 1981;14:550-6.

C-reactive protein: A limited test for managing patients with preterm labor or preterm rupture of membranes?

J. M. Ernest, M.D., Melissa Swain, R.N., Steven M. Block, M.D., Lewis H. Nelson, M.D., Christos G. Hatjis, M.D., and Paul J. Meis, M.D.

Winston-Salem, North Carolina

C-reactive protein has been used to identify patients at high risk for infectious morbidity with preterm labor or preterm rupture of membranes. In this article we report on 104 patients with preterm labor symptoms (n = 45) or preterm rupture of the membranes (n = 59) and serial evaluations of C-reactive protein measured by latex agglutination and laser nephelometry. The simple, inexpensive latex method appears comparable to the laser method in predicting infectious morbidity and can be used clinically. Elevated C-reactive protein values before delivery predict infectious morbidity in only 8% to 29% of patients, and up to 18% of patients with serious infections may be misdiagnosed as having normal C-reactive protein values before delivery. (AM J OBSTET GYNECOL 1987;156:449-54.)

Key words: C-reactive protein, preterm labor, preterm rupture of membranes

Preterm labor and delivery remains the leading cause of perinatal morbidity and mortality today. A major problem associated with preterm labor is intra-amniotic infection, which may occur either before or after rupture of the fetal membranes.[1-4] While these intra-amniotic infections can lead to serious sequelae in both mother and infant, our ability to detect early amnionitis remains poor.

Several authors have recently evaluated the acute-phase reactant C-reactive protein as a potential marker of infectious morbidity in patients at risk for preterm delivery.[5-11]

The purpose of this paper is to report C-reactive protein levels and infectious morbidity in patients with preterm labor symptoms or preterm rupture of the membranes.

Methods and materials

Between August 1, 1983, and May 31, 1984, all patients between 26 and 34 weeks' gestation presenting to the Consolidated Obstetrical Service of Forsyth Memorial Hospital and Wake Forest University Medical Center with preterm rupture of the membranes (n = 114) or signs of preterm labor (n = 171) were considered eligible for inclusion in the study group. After obtaining informed consent as approved by the Clinical Research Practices Committee from 104 patients, each patient had a mean of 8.7 C-reactive protein levels measured (range = 1 to 32) before delivery for a total of 907 samples. Forty-five of the 104 patients had signs of preterm labor, which included uterine contractions, change in cervical dilatation or effacement, or both, while the remaining 59 patients had rupture of the membranes as documented by pH testing and "fern-ing" of vaginal fluid during a sterile speculum examination. Patients with preterm labor documented by uterine contractions and cervical changes were begun on a regimen of intravenous tocolytic agents and changed to oral tocolytic drugs per established protocols. If labor was successfully arrested, preterm labor patients were discharged home to continue oral tocolytics until 37 weeks' gestation. All preterm labor patients discharged undelivered were considered as not having amnionitis, and at subsequent delivery none had signs of amnionitis or neonatal infection. Five patients were admitted on two separate occasions and each admission was considered separately. Patients with documented preterm rupture of the membranes were managed expectantly in the hospital until delivery and were allowed to labor spontaneously unless amnionitis, fetal distress, or fetal lung maturity, documented by a lecithin/sphingomyelin ratio of ≥2:1 or the presence of phosphatidylglycerol in vaginally collected amniotic fluid, was noted, at which time delivery was facilitated by oxytocin induction or cesarean delivery. A total of 109 admissions of the 104 patients who consented to participate were studied.

C-reactive protein was measured daily by two parallel methods, latex agglutination and laser nephelometry.

From the Departments of Obstetrics and Gynecology and Pediatrics, Bowman Gray School of Medicine.

Funded in part by a grant from Behring Diagnostics, a subsidiary of American Hoechst Corporation, La Jolla, California.

Received for publication March 5, 1986; revised July 22, 1986; accepted August 27, 1986.

Reprint requests: J. M. Ernest, M.D., Department of Obstetrics and Gynecology, Bowman Gray School of Medicine, 300 South Hawthorne Road, Winston-Salem, NC 27103.

Table I. Infectious morbidity and latex C-reactive protein positive any time before delivery

	No. of patients per no. of infections	*Test method*	*Sensitivity (%)*	*Specificity (%)*	*Positive predictive value (%)*	*Negative predictive value (%)*	χ^2	*p*
Maternal amnionitis	97/8	Latex	88	44	12	98	1.819	0.17
Maternal amnionitis and/or neonatal infection	97/13	Latex	77	44	18	93	1.269	0.26
Any maternal or neontal infection	109/25	Latex	76	44	29	86	2.457	0.11

Table II. Infectious morbidity and laser C-reactive protein >0.8 mg/dl any time before delivery

	No. of patients per no. of infections	*Test method*	*Sensitivity (%)*	*Specificity (%)*	*Positive predictive value (%)*	*Negative predictive value (%)*	χ^2	*p*
Maternal amnionitis	85/6	Laser	67	38	8	94	0.044	0.67
Maternal amnionitis and/or neonatal infection	85/10	Laser	60	37	11	88	0.034	0.66
Any maternal or neonatal infection	94/19	Laser	68	37	22	82	0.040	0.67

Latex agglutination (Rapitex, Calbiochem-Behring*) is a rapid antigen detection method that is reported as positive (>0.8 mg/dl) or negative (≤0.8 mg/dl) as determined by agglutination on an agitated slide of the serum (C-reactive protein antigen) and reagent (antibody to C-reactive protein). Laser nephelometry quantitatively determines C-reactive protein and reports values from 0.6 to 10 mg/dl. Normal nonpregnant values range from 0.08 to 0.8 mg/dl.[12] There is an excellent correlation between the latex and laser nephelometric C-reactive protein determinations (unpublished data). Neither method was used for patient management. Separate analyses of C-reactive protein values obtained within 12 hours before delivery and C-reactive protein values obtained at any time after hospitalization but before delivery were performed. Both latex (reported as positive or negative) and laser (positive considered to be >0.8 mg/dl and a separate analysis with positive >2.0 mg/dl) values were studied. The laser nephelometry values were chosen because >0.8 mg/dl correlates with a positive latex agglutination test and >2.0 mg/dl is a value reported in the literature as being predictive of infections morbidity.

Definition of infectious morbidity. After other sources of infection were excluded by careful physical examination, routine urinalysis, urine culture and sensitivity when indicated, and other indicated tests (chest x-ray film, for example) and after vaginal culture for group B β-hemolytic streptococci and *Neisseria gonorrhoeae*, amnionitis was considered present by documentation of the following: (1) maternal temperature >38° C (100.4° F) before delivery with any two of the following—white blood cell count >20,000/mm³ in the absence of steroid administration for at least 48 hours before white blood cell count determination, maternal or fetal tachycardia, uterine tenderness, or foul-smelling amniotic fluid; (2) pathogens isolated from the placenta at the time of cesarean delivery; (3) pathogens isolated from amniotic fluid obtained at amniocentesis.

Pathogens from the placenta or amniotic fluid included group B β-hemolytic streptococci, *Acinetobacter*, and anaerobes. Other maternal infections included urinary tract infections (>10^5 colonies per milliliter), recent conversion to positive VDRL test results, otitis media, bacterial pneumonia, and viral upper respiratory infection.

Neonatal congenital infection was diagnosed within 3 days of birth. Criteria for definite sepsis included clinical illness and positive blood cultures. Criteria for probable sepsis included at least two of these criteria: (1) clinical illness; (2) complete blood count with neutropenia or immature to total neutrophil ratio >0.20; (3) positive gastric aspirate, Gram stain, or culture; (4) positive placental culture; (5) foul odor.

Evidence of infection was not considered to be present if positive vaginal or placental cultures for common vaginal flora (for instance, group B streptococci or *Escherichia coli*) were reported after vaginal delivery. Histologic chorioamnionitis was not evaluated because of the uncertain correlation between that parameter and clinical infection.[2, 13]

*Behring Diagnostics, Division of American Hoechst Corporation, 10933 North Torrey Pines Road, La Jolla, CA 92037.

Table III. Infectious morbidity and laser C-reactive protein >2.0 mg/dl any time before delivery

	No. of patients per no. of infections	*Test method*	*Sensitivity (%)*	*Specificity (%)*	*Positive predictive value (%)*	*Negative predictive value (%)*	χ^2	*p*
Maternal amnionitis	85/6	Laser	60	64	10	96	0.420	0.52
Maternal amnionitis and/or neonatal infection	85/10	Laser	30	61	9	87	0.034	0.66
Any maternal or neonatal infection	94/19	Laser	47	61	24	82	0.184	0.65

The sensitivity, specificity, and positive and negative predictive values were determined for C-reactive protein levels in three separate clinical situations: (1) maternal amnionitis alone, (2) maternal amnionitis with or without neonatal infection, (3) maternal amnionitis or any other maternal infection with or without neonatal infection. The χ^2 analysis was used with $p < 0.05$ considered significant.

Results

A total of 27 infectious episodes were noted during the 109 admissions, including eight patients with amnionitis, seven with neonatal infection, and 12 with other maternal infections (eight with urinary tract infections and one each with recent positive VDRL test results, otitis media, bacterial pneumonia, and viral upper respiratory infection. Only two of the eight patients with amnionitis had concomitant neonatal sepsis.

Tables I through III give the sensitivity, specificity, and predictive values for infectious morbidity in patients with preterm labor or with preterm rupture of the membranes with the use of any positive C-reactive protein values at any time before delivery. In Table I C-reactive protein is measured by latex agglutination with values >0.8 mg/dl reported as positive, while Tables II and III report C-reactive protein measured by laser nephelometry with values >0.8 and 2.0 mg/dl, respectively, reported as positive. Sensitivity, specificity, and predictive values with a positive C-reactive protein value within 12 hours before delivery for the latex and laser measurement were also calculated and showed similar results to those of Tables I through III.

The negative predictive value of C-reactive protein measurements is much greater than the positive predictive value in all instances. In general, sensitivity was greater than specificity for most categories considered.

After other sources of infection were ruled out and when maternal amnionitis and neonatal infection only were considered, a positive C-reactive protein value at any time before delivery by either test method predicted infection 9% to 18% of the time. When only C-reactive protein values obtained within 12 hours before delivery were considered, the predictability was not improved and remained between 8% and 19%. These false positive C-reactive protein values, when quantitated by laser nephelometry, ranged from 2.1 to 9.8 mg/dl with no evidence of infection. False negative C-reactive protein values were less common but were present in 7% to 13% of patients with amnionitis or neonatal infection. These patients with "false normal" C-reactive protein values included a neonate with enterococcal sepsis, a neonate with group B streptococcal sepsis, and a patient whose placental cultures obtained at the time of cesarean section grew group B β-hemolytic streptococci.

Comment

Since 1980 seven articles have reported C-reactive protein values and infectious morbidity during pregnancy. Four reported on patients with premature rupture of the membranes,[5,7,8,10] one included patients with preterm labor and premature rupture of the membranes,[6] and two correlated C-reactive protein levels with outcome of tocolysis in patients in preterm labor.[9,11] A summary of these reports is presented in Table IV. A comparison of the sensitivity, specificity, and predictive values from previous studies with those of the present article is presented in Table V.

Six of the seven groups reported that C-reactive protein measurements are useful predictors of infectious morbidity, while Farb et al.[6] stated that C-reactive protein measurements are "not a specific or sensitive indicator of clinical or histologic amnionitis." The present study finds similarities with and differences from previous reports.

As in previous studies, the negative predicitve value of C-reactive protein is greater than the positive predictive value. In the present report, if all C-reactive protein values were negative before delivery, only 7% to 13% of patients would exhibit evidence of maternal amnionitis and/or neonatal infection. If one or more C-reactive protein values were positive before delivery, however, the test could no longer be used as a negative predictor, and only 9% to 18% of patients with one or more positive values would show evidence of maternal amnionitis and/or neonatal infection. This low positive predictive value contrasts to results in the majority of previous articles with reported positive predictive val-

Table IV. Review of previous studies

Series	*No. of patients*	*Type of patients*	*Method of measurement and value considered positive*
Evans et al.,[5] 1980	109 (36 with PROM <36 wk gestation)	Patients with PROM ≤36 wk gestation and controls with spontaneous rupture of membranes at term, preterm labor with intact membranes, and term labor with intact membranes	Laser nephelometry; ≥2.0 mg/dl
Farb et al.,[6] 1983	90	31 PROM and 41 PTL patients between 20 and 36 wk gestation and 18 "high risk"	Laser nephelometry; ≥2.0 mg/dl
Hawrylyshyn et al.,[7] 1983	52 (42 delivered)	PROM <34 wk gestation	Laser nephelometry; >1.25 mg/dl
Romem and Artal,[8] 1984	51	PROM ≤34 wk gestation	Laser nephelometry; >1.78-1.89 mg/dl
Handwerker et al.,[9] 1984	50	Preterm labor 24-34 wk gestation	Latex agglutination and capillary precipitation methods
Ismail et al.,[10] 1985	100	PROM <35 wk	Laser nephelometry; >2 mg/dl
Potkul et al.,[11] 1985	62	40 in preterm labor 24-36 wk and 22 controls	Laser nephelometry; >0.7 mg/dl

PROM = Preterm rupture of the membranes; SLE = systemic lupus erythematosus; RA = rheumatoid arthritis; PTL = preterm labor.

Table V. Comparison of C-reactive protein studies and outcomes reported

	Preterm rupture of membranes and infectous morbidity				*Preterm rupture of membranes, preterm labor, and infectious morbidity*		*Preterm labor and tocolytic success*	
	Evans et al.[5]	*Hawrylyshyn et al.*[7]	*Romem and Artal*[8]	*Ismail et al.*[10]	*Farb et al.*[6]	*Present article**	*Handwerker et al.*[9]	*Potkul et al.*[11]
Sensitivity (%)	81	88	86-100	82	59	30-77	85	91
Specificity (%)	100	96	97-100	55	82	37-61	89	79
Positive predictive value (%)	81	96	86-100	36	50	9-18	73	63
Negative predictive value (%)	100	89	97-100	91	87	87-93	94	96

*Maternal amnionitis and/or neonatal infection.

Definition of infectious morbidity	*Comments*
Any of following present before delivery: Temperature ≥37.5° C, positive maternal or neonatal culture, histologic chorioamnionitis, elevation of white blood cell count to 50% over baseline value, >6 bands on differential, any toxic granulations	Excluded intracurrent illness such as SLE and RA; no mention of steroid use
Clinical: Temperature >37.5° C or white blood cell count rise ≥50% above admission level together with either uterine tenderness or fetal tachycardia; also used histologic criteria	C-reactive protein obtained every 12 hr until delivery; steroids given; "not specific or sensitive indicator of clinical or histologic chorioamnionitis"
Histologic chorioamnionitis; subgroups with temperature ≥38° C within 12 hr of delivery or positive endometrial-placental cultures	Compared C-reactive protein to white blood cell count, erythrocyte sedimentation rate, % bands
Temperature >100° F and other criteria "established by Gibbs, Koh, Garite, and others"	Steroids given; stated "prolonged uterine activity >6 hours" was cause of elevated C-reactive protein
Studied successful or failed tocolysis with positive C-reactive protein	Urinary tract infection not a confounding factor according to authors
Temperature ≥38° C or tender uterus or foul-smelling amniotic fluid and fetal tachycardia; also reviewed histologic chorioamnionitis	—
Studied successful or failed tocolysis and C-reactive protein	—

ues for amnionitis or neonatal infection of 50% to 100%.

There are several possible reasons for the varying results between the present study and previous studies. The present study uses a stricter definition of amnionitis than was used in earlier studies in an attempt to avoid placement of noninfected patients into the infected group. The present definition could therefore exclude some patients with early or subclinical infection. "Histologic chorioamnionitis" was not used to define infection in the present study since previous reports have noted its occurrence in many noninfected patients and its validity as a marker of true infection has been questioned. Fox,[14] for example, found some degree of histologic chorioamnionitis present in 24.4% of 1000 consecutively delivered placentas in Manchester, England. Driscoll[15] observed acute inflammation of amnion and chorion in 10.7% of 3600 cases and could not use placental inflammation to distinguish between infected and noninfected cases. More recently, Bobitt et al.[2] reported no correlation between histologic chorioamnionitis and the presence or absence of microorganisms in amniotic fluid, and Mortimer et al.[13] stated that "placental villitis is usually not an infective condition." White blood cell count elevations within 48 hours of maternal steroid administration were not used to define infectious morbidity since leukocytosis is commonly seen without infection after systemic steroid use. Disease incidence can affect the predictive accuracy of a test and should be examined when studies with different results are compared. The percent of patients with evidence of histologic chorioamnionitis in the previously reported studies ranged from 17% to 63% with a mean of 37% while the clinical chorioamnionitis rates were between 13% and 47% with a mean of 25%. The present study had comparable rates of overall infectious morbidity of 25% and amnionitis or neonatal infection rates of 12%. The differences in incidence between studies may explain slight variations in predictive value but should not account for the wide variation between positive predictive values between the present and previous reports.

In conclusion, an early simple marker of occult intraamniotic infection in patients at risk for infectious morbidity would be very useful in the management of certain high-risk obstetrics patients. Expectantly managed patients with premature rupture of the membranes as well as patients in preterm labor would benefit from additional parameters of infection that could be used in conjunction with the maternal temperature, maternal and fetal heart rates, uterine tenderness, and white blood cell count and differential report. In contrast to results of previous reports, a patient with all normal C-reactive protein values before delivery in whom other causes of infection have been ruled out may still have a 13% chance of developing maternal amnionitis or a neonatal infection while a positive (elevated) C-reactive protein value will predict amnionitis or neonatal infection only 9% to 18% of the time.

If C-reactive protein levels are to be used in the management of patients in preterm labor or with premature rupture of the membranes, the latex agglutination test is as reliable as the more expensive and complicated laser nephelometric determination. C-reactive protein values may be helpful if consistently normal (or negative), but significant maternal or neonatal infection may still occur in up to one of eight of those patients. Elevated C-reactive protein values predict infection in only one of five patients and are therefore inaccurate predictors of infectious morbidity.

REFERENCES

1. Minkoff H. Prematurity: infection as an etiologic factor. Obstet Gynecol 1983;62:137.
2. Bobitt JR, Hayslip CC, Damato JD. Amniotic fluid infection as determined by transabdominal amniocentesis in patients with intact membranes in premature labor. AM J OBSTET GYNECOL 1981;140:947.
3. Naeye RL, Peters EC. Amniotic fluid infections with intact membranes leading to perinatal death: a prospective study. Pediatrics 1978;61:171.
4. Garite TJ, Freeman RK. Chorioamnionitis in the preterm gestation. Obstet Gynecol 1982;59:539.
5. Evans MI, Hajj SN, Devoe LD, Angerman NS, Moawad AH. C-reactive protein as a predictor of infectious morbidity with premature rupture of membranes. AM J OBSTET GYNECOL 1980;138:648.
6. Farb HF, Arnesen M, Geistler P, Knox GE. C-reactive protein with premature rupture of membranes and premature labor. Obstet Gynecol 1983;62:49.
7. Hawrylyshyn P, Bernstein P, Milligan JE,, Soldin S, Pollard A, Papsin FR. Premature rupture of membranes: the role of C-reactive protein in the prediction of chorioamnionitis. AM J OBSTET GYNECOL 1983;147:240.
8. Romem Y, Artal R. C-reactive protein as a predictor for chorioamnionitis in cases of premature rupture of the membranes. AM J OBSTET GYNECOL 1984;150:546.
9. Handwerker SM, Tejani NA, Verma UL, Archbald F. Correlation of maternal serum C-reactive protein with outcome of tocolysis. Obstet Gynecol 1984;63:220.
10. Ismail MA, Zinaman MJ, Lowensohn RI, Moaward AH. The significance of C-reactive protein levels in women with premature rupture of membranes. AM J OBSTET GYNECOL 1985;151:541.
11. Potkul RK, Moaward AH, Ponto KL. The association of subclinical infection with preterm labor: the role of C-reactive protein. Obstet Gynecol 1985;153:642.
12. Pepys MB. C-reactive protein fifty years on. Lancet 1981;1:653.
13. Mortimer G, MacDonald DJ, Smeeth A. A pilot study of the frequency and significance of placental villitis. Br J Obstet Gynaecol 1985;92:629.
14. Fox H. Infections of the placenta. In: Coid CR, ed. Infections and pregnancy. London: Academic Press, 1977: 255.
15. Driscoll SG. The placenta and membranes. In: Charles D, Finland M, eds. Philadelphia: Lea & Febiger, 1973:532.

Comparison of phospholipids in vaginal and amniocentesis specimens of patients with premature rupture of membranes

David C. Shaver, M.D., Joseph A. Spinnato, M.D., David Whybrew, M.S., W. Keith Williams, M.D., and Garland D. Anderson, M.D.

Memphis, Tennessee

Amniotic fluid was obtained from the vaginal pool and by transabdominal amniocentesis in 28 patients who were admitted to the hospital between 26 and 35 weeks' gestation with premature rupture of the membranes. Phospholipid profiles were obtained on all specimens with the use of high-performance liquid chromatography. The lecithin/sphingomyelin ratio, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine, and lysolecithin were all analyzed to determine the effect of vaginal contamination. With the use of a paired *t* test, only lysolecithin was shown to be significantly affected by vaginal contamination ($p < 0.05$). As determined by high-performance liquid chromatography, evaluation of phospholipids in vaginal fluid of patients with premature rupture of the membranes appears to be an accurate and reliable method for predicting fetal lung maturity. (AM J OBSTET GYNECOL 1987; 156:454-7.)

Key words: Premature rupture of membranes, lecithin/sphingomyelin ratio, pulmonary phospholipids

Neonatal pulmonary performance is a major determinant of ultimate outcome for the prematurely delivered infant. Suggested management strategies for patients with premature labor have relied heavily on the presence or absence of fetal pulmonary maturity, as determined by assessment of amniotic fluid obtained through amniocentesis. However, amniocentesis for such purposes in patients with premature rupture of the membranes is possible in only 50% of cases.[1] Although transvaginally collected amniotic fluid has been used for management decisions in some series, the reliability of these determinations has been questioned. The purpose of this prospective study is to compare phospholipid measurements from amniotic fluid specimens obtained vaginally with those obtained transab-

From the Division of Maternal/Fetal Medicine, Department of Obstetrics and Gynecology, University of Tennessee.
Received for publication May 20, 1986; revised July 25, 1986; accepted August 14, 1986.
Reprint requests: David C. Shaver, M.D., Department of Obstetrics and Gynecology, Division of Maternal/Fetal Medicine, 800 Madison Ave., Memphis, TN 38163.

Table I. Results of lecithin/sphingomyelin ratio and phosphatidylglycerol determination in both vaginal and amniocentesis specimens

Patient No.	*Gestational age (wk)*	*Lecithin/sphingomyelin ratio*		*Phosphatidylglycerol*	
		Amniocentesis	*Vaginal*	*Amniocentesis*	*Vaginal*
1	30	1.93	2.13	+	+
2	33	2.95	3.09	−	−
3	33	3.02	3.36	−	−
4	26	.57	.60	−	−
5	34	2.02	1.90	+	+
6	29	3.09	3.08	−	−
7	30	1.12	1.28	−	−
8	32	1.41	1.83	−	−
9	33	1.89	2.10	−	−
10	35	4.94	5.18	+	+
11	28	1.72	1.43	−	−
12	35	2.64	3.15	+	+
13	33	3.42	7.13	−	−
14	33	3.43	4.12	−	−
15	35	4.60	5.22	+	+
16	34	2.05	2.14	+	+
17	30	1.50	1.28	−	−
18	34	2.21	2.39	+	+
19	30	1.05	1.40	−	−
20	32	2.35	2.56	−	−
21	32	2.27	2.16	−	−
22	34	2.16	2.14	+	+
23	33	2.25	2.62	−	−
24	28	1.44	1.17	−	−
25	35	1.93	1.96	−	−
26	29	1.75	1.67	−	−
27	33	2.83	2.82	−	−
28	34	1.86	1.94	+	+

dominally in patients with preterm premature rupture of the membranes.

Material and methods

Fluid collection. Approximately 100 patients who were admitted to the E. H. Crump Women's Hospital and Perinatal Center with the diagnosis of premature rupture of the membranes were evaluated for inclusion in the study. Only patients with gestational ages ranging from 26 to 35 weeks were considered. Following admission to the hospital, a sterile speculum examination was performed to confirm premature rupture of the membranes, and amniotic fluid was collected from the posterior fornix. Free-flowing fluid from the cervix was not required; in several cases only pooled vaginal fluid could be obtained. Ultrasonography was then performed, and if an adequate pocket of fluid was identified an amniocentesis was carried out under aseptic conditions, with real-time ultrasound guidance. If no site for amniocentesis could be identified, the patient was placed in the Trendelenburg position and reevaluated in several hours. If timely amniocentesis could not be performed, the patient was excluded. All vaginal and amniocentesis specimens were obtained within 12 hours of each other. Because of difficulty in obtaining both vaginal and amniocentesis fluid in each patient, 28 patients who fulfilled these criteria were enrolled in the study.

Instrumentation. All amniotic fluid phospholipid separations were carried out on a Hewlett-Packard Model 1084B high-performance liquid chromatograph equipped with a variable wavelength detector. The chromatographic separation was achieved with the use of a 5 cm, 40 μm Porasil (Supelco, Inc.) guard column followed with a 20 cm by 4.6 mm LiChrosorb 10 μm Diol column (Hewlett-Packard). The eluted phospholipids were quantitated by ultraviolet absorption at 203 nm and the detector response set at 0.0128 au/cm.

Analytical conditions. A linear gradient was run from 2.7% to 15% water in acetonitrile. The separation of the phospholipids was achieved at a flow rate of 1.2 ml/min. The solvent temperatures were maintained at 40° C, while the column temperature was 35° C. Integration of the peak areas was the means of quantitation.

Chromatographic analysis. Standard curves for each of the phospholipids of interest were determined by assaying 3, 5, 10, 15, and 20 μg amounts of each phospholipid in 5 μl quantities. Each of the phospholipid peaks was verified by thin-layer chromatography according to the technique of Hallman et al.[2] Amniotic fluid phospholipids were extracted by the procedure of Morrison et al.,[3] except that the amniotic fluid sam-

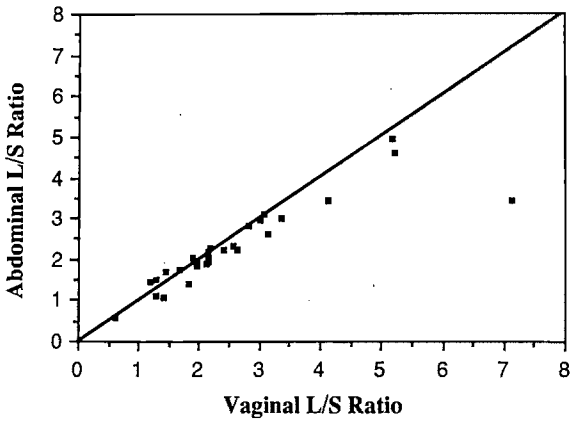


Fig. 1. Scatterplot showing the relationship between lecithin/sphingomyelin *(L/S)* ratios of vaginal and amniocentesis fluid.

ples were centrifuged at 1000 × g. The dried extracted material was dissolved in 30 μl of chloroform and then assayed with high-performance liquid chromatography.

Statistical methods. Statistical evaluations were performed with the use of the paired *t* test and correlation coefficient. A p value of <0.05 was considered statistically significant.

Results

In all 28 patients included in the study, at least 5 ml of both vaginal and amniocentesis fluid was obtained. None were contaminated with blood or meconium.

High-performance liquid chromatography. The lecithin/sphingomyelin ratios, presence or absence of phosphatidylglycerol, and gestational age at the time of sampling for each patient are shown in Table I. In 25 of 28 patients (89%), both vaginal and amniocentesis specimens were in agreement in regard to maturity ($\geq$2.0:1) or immaturity ($<$2.0:1) of lecithin/sphingomyelin ratios. In the three remaining patients (Patients 1, 5, and 9) the lecithin/sphingomyelin ratios were closely paired slightly above or below a ratio of 2 in each case. The lecithin/sphingomyelin ratios are represented graphically in Fig. 1. As can be seen, there is good correlation between the samples, with a correlation coefficient of 0.88. The mean lecithin/sphingomyelin ratio of amniocentesis fluid was 2.3:1 (±0.99), and the mean lecithin/sphingomyelin ratio of vaginal fluid was 2.56 (±1.41). Paired *t* test did not reveal a significant difference between the specimens ($p = 0.06$).

Phosphatidylglycerol was present in amniocentesis specimens of nine patients. All patients with positive test results for phosphatidylglycerol, with one exception (Patient 1, 30 weeks), were at $\geq$34 weeks' gestation. Each patient with amniocentesis specimens containing phosphatidylglycerol also had clearly detectable phosphatidylglycerol in vaginal fluid. The correlation coefficient for phosphatidylglycerol was 0.94, and paired *t* test demonstrated no significant difference between the two groups ($p > 0.2$).

Comparisons of phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine, and lysolecithin were also done. Correlation coefficients and results of paired *t* test of all the phospholipids measured are noted in Table II. Lysolecithin was the only phospholipid that was demonstrated to be significantly affected by vaginal contamination.

Neonatal outcome. None of the infants in cases where either vaginal or amniocentesis fluid indicated fetal lung maturity (18 patients) experienced respiratory complications. In the 10 patients with immature lecithin/sphingomyelin ratios, four infants (40%) had respiratory complications. Two of these infants were diagnosed as having the respiratory insufficiency of prematurity, and one of them died (Patient 4). The other two carried a diagnosis of respiratory distress syndrome and one of these infants also died (Patient 24). The incidence of pulmonary complications is of limited significance, however, since tocolytics and steroids were administered to several patients, and delivery was delayed for one week in three patients.

Comment

Assessment of fetal lung maturity is usually included in the evaluation of patients in whom premature delivery is contemplated or in those who are felt to be at high risk for premature delivery. However, in virtually all studies on management of patients with premature rupture of the membranes, no assessment of fetal lung maturity is included. This is presumably due to an unwillingness to accept the reliability of such studies on fluid obtained vaginally. An exception to this is the presence of phosphatidylglycerol in vaginal fluid, which has been shown to be an accurate predictor of fetal lung maturity.[4, 5]

This reluctance to accept the accuracy of studies on fluid obtained vaginally apparently dates from early work done on fetal lung maturity. Although several reports since that time have disputed these conclusions, they remain entrenched in the literature and in management practices.

Most reports that have addressed the issue of vaginal contamination in studies of lung maturity have not included patients with premature rupture of the membranes. Instead, several approaches such as washings of the vagina or transcervical amniocentesis with subsequent amniotomy have been used. Additionally, nearly all patients who were evaluated were at term gestation, and therefore the incidence of immature lecithin/sphingomyelin ratios was quite low.

Several reports have indicated a good correlation of

Table II. Relationship between vaginal and amniocentesis specimens of amniotic fluid

Phospholipid	*Amniocentesis specimen (mean ± SD)*	*Vaginal specimen (mean ± SD)*	*Correlation coefficient (r)*	*Paired t test (p value)*
Lecithin/sphingomyelin	2.3 ± 0.987	2.56 ± 1.41	0.88	0.06
Phosphatidylglycerol	0.045 ± 0.098	0.049 ± 0.098	0.94	0.20
Phosphatidylinositol	0.199 ± 0.156	0.190 ± 0.186	0.69	>0.20
Phosphatidylethanolamine	0.362 ± 0.247	0.365 ± 0.203	0.89	>0.20
Phosphatidylserine	0.293 ± 0.199	0.292 ± 0.205	0.88	>0.20
Lysolecithin	0.08 ± 0.123	0.112 ± 0.139	0.83	<0.05

lecithin/sphingomyelin ratios in amniotic fluid before and after vaginal contamination. Sbarra et al.[6] demonstrated that lavage of the vagina did not affect the lecithin/sphingomyelin ratio and further showed that there was no detectable lecithin or sphingomyelin in washings of vaginal fluid. Similar findings regarding lecithin/sphingomyelin ratios were reported by Phillippe et al.[7] and Golde.[8]

Dombroski et al.[9] evaluated 27 patients at term with transvaginal amniocentesis and subsequent fluid collection after amniotomy. Although lecithin/sphingomyelin ratio and phosphatidylglycerol correlated well, they expressed concern because the vaginal lecithin/sphingomyelin ratio exceeded the amniocentesis result 22% of the time.

All of the patients included in this study were <36 weeks' gestation with premature rupture of the membranes and with a wide range of lecithin/sphingomyelin ratios demonstrated. As measured by high-performance liquid chromatography, there appears to be little effect of vaginal contamination on the phospholipids commonly used to assess fetal lung maturity. Although the difference between lecithin/sphingomyelin ratios did approach statistical significance ($p = 0.06$) because of a slightly higher mean lecithin/sphingomyelin ratio in the vaginal group, this had virtually no effect on the individual patients and should be of little clinical significance. In only one case (Patient 13) was there a marked discrepancy in lecithin/sphingomyelin ratios (3.42:1 versus 7.13:1), which would have no impact on management.

The present study confirmed the reliability of assessing vaginal fluid for phosphatidylglycerol. However, phosphatidylglycerol was present in a minority of patients (nine of 28, or 32%), which would be expected at the gestational ages that were represented. Therefore, the ability to use other parameters of fetal pulmonary maturity, such as lecithin/sphingomyelin ratio, assumes greater importance.

Confidence in the accuracy of pulmonary maturity studies of vaginally obtained amniotic fluid should have an impact on management of patients with premature rupture of the membranes. Decisions regarding subjects such as the use of tocolytics, administration of corticosteroids, or timing of induction of labor could all be made with more authority if the status of fetal lung maturity were known.

REFERENCES

1. Garite TJ, Freeman RK, Linzey EM, Braly P. The use of amniocentesis in patients with premature rupture of the membranes. Obstet Gynecol 1979;45:226.
2. Hallman M, Kulovich M, Kirkpatrick E, et al. Phosphatidylinositol and phosphatidylglycerol in amniotic fluid: indices of lung maturity. Am J Obstet Gynecol 1976; 125:613-7.
3. Morrison JC, Wiser WL, Arnold SW, et al. Modification of the lecithin/sphingomyelin assay for fetal development. Am J Obstet Gynecol 1974;120:1087-91.
4. Stedman CM, Crawford S, Staten E, Cherney WB. Management of preterm premature rupture of membranes: assessing amniotic fluid in the vagina for phosphatidylglycerol. Am J Obstet Gynecol 1981;140:34-8.
5. Brame RG, MacKenna J. Vaginal pool phospholipids in the management of premature rupture of membranes. Am J Obstet Gynecol 1983;145:992-1000.
6. Sbarra AJ, Blake G, Cetrulo CL, et al. The effect of cervical/vaginal secretions on measurements of lecithin/sphingomyelin ratio and optical density at 650 nm. Am J Obstet Gynecol 1981;139:214-6.
7. Phillippe M, Acker D, Torday J, Schiff I, Frigoletto FD. The effects of vaginal contamination on two pulmonary phospholipid assays. J Reprod Med 1982;5:283-6.
8. Golde SH. Use of obstetric perineal pads in collection of amniotic fluid in patients with rupture of the membranes. Am J Obstet Gynecol 1983;146:710-2.
9. Dombroski RA, MacKenna J, Brame RG. Comparison of amniotic fluid lung maturity profiles in paired vaginal and amniocentesis specimens. Am J Obstet Gynecol 1981; 140:461-4.

Acute *Haemophilus influenzae* chorioamnionitis associated with intact amniotic membranes

Hung N. Winn, M.D., and Charles C. Egley, M.D.
Peoria, Illinois

While *Haemophilus influenzae* infection commonly occurs in the age group 6 to 48 months, it uncommonly affects adults. A case of acute chorioamnionitis due to *Haemophilus influenzae* in the presence of intact fetal membranes is presented. The low incidence of maternal urogenital carriage, the high attack rate, and the virulence are discussed. (Am J Obstet Gynecol 1987;156:458-9.)

Key words: Chorioamnionitis, sepsis, antibiotics

Acute chorioamnionitis occasionally occurs in the presence of intact fetal membranes. Premature rupture of the membranes significantly increases the risk of acute amnionitis. While a number of organisms have been associated with chorioamnionitis, *Haemophilus influenzae* has been a rare isolate. Gibson and Williams[1] reported the first case of *Haemophilus influenzae* amnionitis associated with premature rupture of the membranes. Reviewing the obstetric literature, we believe this is the first report of *Haemophilus influenzae* causing acute amnionitis while the membranes are still intact.

Case report

The patient, a 25-year-old white married woman, gravida 4, para 3, was transferred to our hospital because of preterm labor at 22 weeks of gestation without ruptured membranes. The patient's prenatal course was remarkable for chills, diarrhea, and nausea for 3 days before admission. Uterine contractions started about 6 hours before the transfer. Tocolysis was initiated with intravenous ritodrine to avoid delivery during transportation. Shortly afterward, ritodrine was discontinued because the patient developed hypotension (systolic and diastolic pressures ranged from 80 to 90 and 30 to 40 torr, respectively), diaphoresis, and a decreased level of consciousness. She had no chest pain or dyspnea. She received a fluid challenge of 1000 ml of 5% dextrose in water and lactated Ringer's solution during transport. On admission to our hospital the patient was conscious and diaphoretic. She had a rectal temperature of 38.9° C and a blood pressure of 90/30 torr in a supine position. Examination revealed regular uterine contractions every 4 to 5 minutes, mild uterine tenderness, and copious purulent discharge from the cervix. Ferning test of the vaginal discharge was negative for amniotic fluid. A tentative diagnosis of sepsis and preterm labor secondary to acute amnionitis was made. Amniocentesis revealed many gram-negative bacilli. A chest roentgenogram showed a left lower lobe infiltration suggestive of pneumonia on admission. The patient was given intravenous ampicillin, clindamycin, and gentamicin. She also received intravenous dopamine infusion at a low dose (10 μg/kg/min) to maintain systolic blood pressure above 90 torr. As soon as her condition had been stabilized, the patient underwent a hysterotomy for delivery of the fetus since the cervix remained closed and thick. A live-born 400 gm male infant with Apgar scores of 1 and 5 at 1 and 5 minutes, respectively, was delivered. The infant died shortly afterward because of immaturity.

Quantitative cultures of amniotic fluid and blood for aerobes and anaerobes revealed *Haemophilus influenzae*, not type B, as a single pathogen, which was sensitive to ampicillin. Cleocin and gentamicin were discontinued on the second postoperative day. The patient was maintained on a regimen of intravenous ampicillin for 5 days and was discharged on a regimen of oral amoxicillin. Intravenous dopamine was discontinued when the patient was in the recovery room. The temperature returned to normal within 24 hours of admission and she remained afebrile until discharged. Her postoperative course was uneventful. A repeat chest roentgenogram showed a decrease in the lung infiltration 3 days later. Serum cold agglutinin and mycoplasma serologic tests were negative. The placental pathologic examination revealed acute chorioamnionitis and funisitis.

Comment

Haemophilus influenzae is a gram-negative, nonmotile aerobic bacillus that requires hematin and nicotinamide adenine dinucleotide or precursor for optimal growth. It is a major cause of meningitis in children from age 3 months to 3 to 6 years. In adults symptomatic infection, when it occurs, often presents as pneumonia, usu-

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Illinois College of Medicine at Peoria.
Received for publication February 28, 1986; revised May 12, 1986; accepted August 14, 1986.
Reprint requests: Hung N. Winn, M.D., Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Illinois College of Medicine at Peoria, P. O. Box 1649, Peoria, IL 61656.

ally from nonencapsulated strains. However, infection due to *Haemophilus influenzae* is uncommon in adults. *Haemophilus influenzae* amnionitis in the presence of intact membranes is especially rare.

There are two possible mechanisms by which *Haemophilus influenzae* can enter the amniotic cavity: hematogenous dissemination from another source of infection or ascending assault from the cervix and vagina. Respiratory infections are important sources of hematogenous spread due to *Haemophilus influenzae* infection. Furthermore, *Haemophilus influenzae* can cause bacteremia even without localized infection. It is possible that our patient had pneumonia due to *Haemophilus influenzae* with subsequent bacteremia and hematogenous dissemination to the amniotic cavity.

Haemophilus influenzae can also enter the amniotic cavity by an ascending route from the vagina and cervix. The carriage rate of this organism in the female genital tract is very low.[2] On the other hand, *Haemophilus influenzae* appears to have a high attack rate and virulence associated with significant maternal and neonatal morbidity. Beargie[2] obtained two positive cultures for *Haemophilus influenzae* among 1083 routine vaginal cultures done on asymptomatic patients admitted to the labor ward. One of the two patients with positive vaginal cultures for *Haemophilus influenzae* developed acute amnionitis and neonatal sepsis.[2] Our patient and the one reported by Gibson and Williams[1] also developed moderate hypotension and high fever early in the disease process. It is possible, although unlikely, that our patient developed two separate diseases at the same time, acute amnionitis from ascending infection and left lower lobe pneumonia.

The drug of choice for *Haemophilus influenzae* is ampicillin. Occasionally, an ampicillin-resistant organism may be isolated, in which case chloramphenicol is the alternative.

In conclusion, since acute amnionitis due to *Haemophilus influenzae* is a rare event, a high index of suspicion is required to diagnose and initiate a timely and appropriate treatment to decrease maternal and neonatal morbidity. The usual triple antibiotic therapy (ampicillin, clindamycin, and gentamicin) remains an excellent choice for acute amnionitis, especially in cases where sepsis is also present. *Haemophilus influenzae* should be added to the list of potential pathogens that can cause acute amnionitis even in the presence of intact membranes. Routine screening for this organism does not seem to be cost-effective at this time in view of the very low carriage rate.

REFERENCES

1. Gibson M, Williams PP. Hemophilus influenzae amnionitis associated with prematurity and premature membrane rupture. Obstet Gynecol 1978;52(suppl):70-2.
2. Beargie R. Perinatal infection and vaginal flora. AM J OBSTET GYNECOL 1975;122:31-3.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1987 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 156 (January-June) and Vol. 157 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Maternal serum α-fetoprotein, age, and Down syndrome risk

Glenn E. Palomaki, B.S., and James E. Haddow, M.D.
Scarborough, Maine

Before the discovery that an association existed between low maternal serum α-fetoprotein levels and Down syndrome, the ability to detect Down syndrome pregnancies was limited to women aged ≥35, whose individual risks were sufficiently high (≥1:270 in the second trimester) to justify offering amniocentesis. If such women were to opt for that procedure, about 20% of all cases of Down syndrome could be detected. It is now possible to identify an additional 20% of all Down syndrome pregnancies in women under age 35, with the use of a screening process that combines a woman's maternal serum α-fetoprotein level and her age. We present a method whereby a woman's individual odds for Down syndrome can be calculated by combining the maternal serum α-fetoprotein measurement with her age, on the basis of published age-related Down syndrome risk data and on maternal serum α-fetoprotein distributions for unaffected and Down syndrome pregnancies. These individual odds calculations provide the basis both for establishing maternal serum α-fetoprotein/Down syndrome screening cutoffs and for counseling. (AM J OBSTET GYNECOL 1987;156:460-3.)

Key words: Down syndrome, maternal serum α-fetoprotein, maternal age, odds, screening

The association between low maternal serum α-fetoprotein levels and Down syndrome has been thoroughly documented.[1-9] Maternal serum α-fetoprotein has been proposed as a screening test for Down syndrome risk,[2,5] and one prospective study has been published that demonstrates the feasibility of such screening.[10] In this paper we describe a method for calculating a woman's individual odds for carrying a Down syndrome pregnancy that takes both her MSAFP level and age into account. Attention has been called to the need for odds tables that can be derived from this method for use by health professionals such as obstetricians and genetic counselors.[11]

Background data necessary for the method

Down syndrome risk estimation by maternal age alone. Table I gives rates of Down syndrome births by maternal age from three sources as reported by Hook,[12] along with the mean rate and the resulting Down syndrome odds at term and midtrimester. The midtrimester odds are based on estimated spontaneous Down syndrome fetal losses of 29% between the second trimester and term.[13]

Distributions of maternal serum α-fetoprotein multiples of the median for unaffected singleton and Down syndrome pregnancies. Fig. 1 shows distributions of the maternal serum α-fetoprotein multiples of the median for both Down syndrome and unaffected singleton pregnancies. Both of these populations fit a log Gaussian distribution[1,2,14]; their log means and standard deviations are estimated from published medians and interquartile ranges.[2] The unaffected singleton distribution has been modified to approximate a population whose dates have been confirmed by sonographic biparietal diameter measurements.[15]

Method

Deriving an individual woman's likelihood ratio for Down syndrome on the basis of maternal serum α-fetoprotein multiples of the median. For an individual woman, the likelihood ratio for a Down syndrome pregnancy versus an unaffected singleton pregnancy can be estimated by first locating the maternal serum α-fetoprotein multiple of the median on the horizontal scale in Fig. 1 and then measuring the vertical distance from that point on the baseline to the Down syndrome and unaffected singleton curves. The likelihood ratio is the distance to the Down syndrome curve divided by the distance to the unaffected singleton curve. For example, the *dashed vertical line* in Fig. 1 indicates a maternal serum α-fetoprotein test result of 0.50 multiple of the median. The distance to the Down syndrome curve is 2.37 times greater than to the unaffected curve. Thus the woman's odds of carrying a Down syndrome fetus are found to be 2.37 times greater than before the maternal serum α-fetoprotein test result was known. The formula for calculating the height (Y) of a Gaussian curve at a given position on the baseline (X is $\log_{10}$ multiple of the median) is expressed in the following equation:

$$Y = \frac{1}{\sqrt{2\pi}\, s} e^{\frac{-(X-u)^2}{2s^2}}$$

From the Foundation for Blood Research.
Received for publication March 11, 1986; accepted August 19, 1986.
Reprint requests: Glenn E. Palomaki, Foundation for Blood Research, P. O. Box 190, Scarborough, ME 04074.

Table I. Estimates of Down syndrome rates and odds both at term and during the second trimester by maternal age

	Down syndrome rate/1000 at term				*Odds*	
Maternal age at term (yr)	*Mass. study*	*New York study*	*Swedish study*	*Mean rate*	*Term*	*Midtrimester*
20	0.57	0.52	0.64	0.58	1:1734	1:1231
21	0.60	0.59	0.67	0.62	1:1612	1:1145
22	0.64	0.65	0.71	0.67	1:1500	1:1065
23	0.67	0.71	0.75	0.71	1:1408	1:1000
24	0.70	0.77	0.79	0.75	1:1327	1:942
25	0.74	0.83	0.84	0.80	1:1250	1:887
26	0.77	0.89	0.87	0.84	1:1186	1:842
27	0.80	0.95	0.92	0.89	1:1124	1:798
28	0.84	1.01	0.97	0.94	1:1064	1:755
29	0.87	1.07	1.02	0.99	1:1014	1:721
30	0.90	1.13	1.08	1.04	1:965	1:685
31	0.93	1.21	1.14	1.09	1:915	1:650
32	1.15	1.38	1.25	1.26	1:794	1:563
33	1.55	1.69	1.47	1.57	1:637	1:452
34	1.98	2.15	1.92	2.02	1:496	1:352
35	2.53	2.74	2.51	2.59	1:386	1:274
36	3.22	3.49	3.28	3.33	1:300	1:213
37	4.11	4.45	4.28	4.28	1:234	1:166
38	5.24	5.66	5.60	5.50	1:182	1:129
39	6.68	7.21	7.32	7.07	1:141	1:100
40	8.52	9.19	9.57	9.09	1:110	1:78
41	10.86	11.71	12.51	11.69	1:86	1:61
42	13.85	14.91	16.36	15.04	1:66	1:47
43	17.66	19.00	21.39	19.35	1:52	1:37
44	22.51	24.20	27.96	24.89	1:40	1:29
45	28.71	30.84	36.55	32.03	1:31	1:22
46	36.61	39.28	47.79	41.23	1:24	1:17
47	46.68	50.04	62.47	53.06	1:19	1:13
48	59.52	63.75	81.67	68.31	1:15	1:10
49	75.89	81.21	106.76	87.95	1:11	1:8

Table II. Likelihood ratio of Down syndrome versus unaffected singleton pregnancy by maternal serum α-fetoprotein multiple of the median

Multiple of the median	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
0.3	—	—	—	—	—	5.19	4.86	4.56	4.29	4.04
0.4	3.81	3.61	3.42	3.25	3.09	2.95	2.81	2.69	2.57	2.47
0.5	2.37	2.27	2.19	2.10	2.03	1.96	1.89	1.83	1.77	1.71
0.6	1.66	1.61	1.56	1.52	1.47	1.43	1.39	1.36	1.32	1.29
0.7	1.26	1.23	1.20	1.17	1.15	1.12	1.10	1.07	1.05	1.03
0.8	1.01	0.99	0.97	0.95	0.93	0.92	0.90	0.89	0.87	0.86
0.9	0.84	0.83	0.82	0.80	0.79	0.78	0.77	0.76	0.75	0.73
1.0	0.72	0.64	0.57	0.52	0.48	0.45	0.42	0.40	0.38	0.36

*To find the likelihood ratio associated with an individual woman's maternal serum α-fetoprotein multiple of the median (for example, 0.50), identify the first significant digit (5) in the left-hand column and match with the second significant digit (0) located in the top row. The likelihood ratio for this example is 2.37.

where u is the mean of a population and s is the associated standard deviation. Table II displays likelihood ratios for Down syndrome after maternal serum α-fetoprotein testing as derived from the formula, in increments of 0.01 from 0.35 to 1.0 multiple of the median and in increments of 0.1 from 1.0 to 1.9 multiples of the median.

Estimating an individual woman's odds for Down syndrome, on the basis of age and maternal serum α-fetoprotein multiples of the median. Individualized odds can be assigned by combining the maternal age odds in Table I with the likelihood ratios in Table II. For example, a 32-year-old woman with a maternal serum α-fetoprotein multiple of the median of 0.50 is first assigned a maternal age odds in the second trimester of 1:563. Then this odds estimate is multiplied by

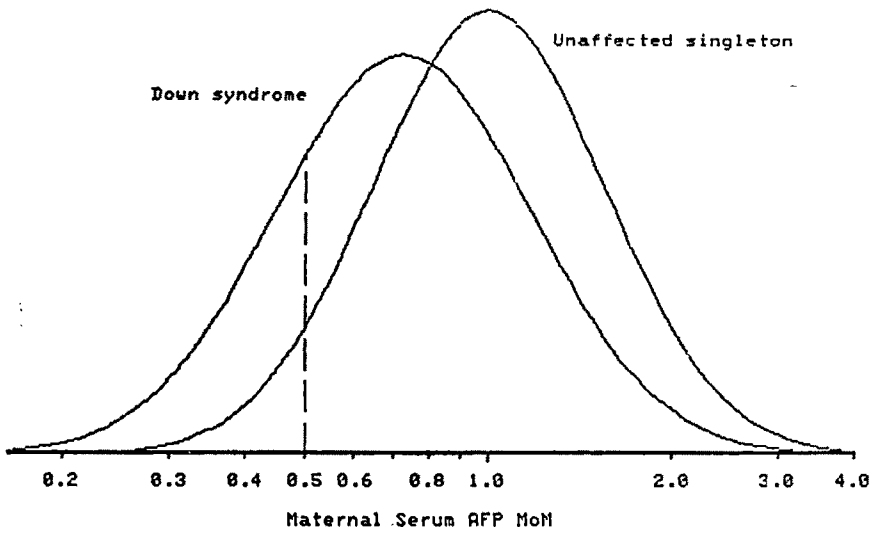


Fig. 1. Plot of the log Gaussian distributions of Down syndrome pregnancies (mean −0.14, SD 0.21) and unaffected singleton pregnancies with ultrasound dating (mean 0.00, SD 0.19).

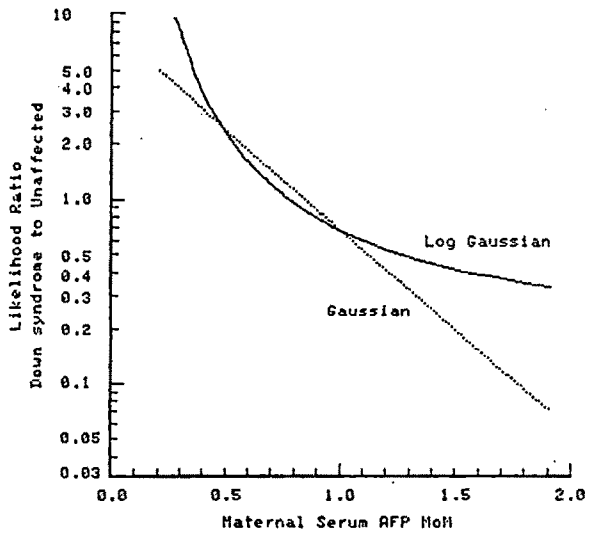


Fig. 2. Plot of the likelihood ratio of Down syndrome versus unaffected singleton pregnancy based on the log Gaussian model and the Gaussian model proposed by Baumgarten.

the likelihood ratio (2.37) associated with the maternal serum α-fetoprotein result, giving a combined odds of 2.37:563 or 1:238.

Comment

Means and standard deviations of the Down syndrome and unaffected singleton distributions have been estimated in this paper from published reports. As more data accumulate, better estimates may appear, and the odds table may need to be updated accordingly. However, the method of odds calculations will remain applicable.

Baumgarten[16] has suggested a method for estimating a woman's individual odds of carrying a Down syndrome fetus based on Gaussian rather than log Gaussian distributions for both the Down syndrome and unaffected singleton populations; in his method, distributions are also assigned identical standard deviations. His method and the standard method described here give similar likelihood ratios between 0.50 and 1.1 multiple of the median (Fig. 2), but they differ at lower and higher multiples of the median. For example, at 1.5 multiples of the median, the likelihood ratio according to the standard method is 0.45, whereas for the method suggested by Baumgarten it is 0.21. Because distributions for the two populations are known to be log Gaussian, risk estimates in the present paper are more likely to be correct.

The maternal serum α-fetoprotein multiple of the median is derived from a combination of gestational age estimation, the maternal serum α-fetoprotein measurement itself, and the laboratory's reference values (medians). As a refinement, the maternal serum α-fetoprotein multiple of the median can be corrected for maternal weight.[17] In our experience, when gestational dates have been calculated from the last menstrual period, about 75% of the maternal serum α-fetoprotein values <0.25 multiple of the median and 50% of the values between 0.25 and 0.50 multiple of the median are associated with overestimated gestational age. For that reason, last menstrual period estimates need to be confirmed by ultrasound before a final risk estimate can be made. Assay performance at the low end of the maternal serum α-fetoprotein range can be a problem[18] because maternal serum α-fetoprotein assays for pregnancy screening were originally optimized in the higher range for neural tube defect identification. Improperly

established maternal serum α-fetoprotein medians can also contribute substantially to error in Down syndrome odds assignment.

At present, pregnant women in the United States aged 35 years and older are offered amniocentesis for Down syndrome detection as part of routine prenatal care. This group of women has a second-trimester Down syndrome risk of at least 1:270 (Table I), and if all these women were to opt for amniocentesis, approximately 20% of all Down syndrome pregnancies could be detected. The association between maternal serum α-fetoprotein and Down syndrome provides an opportunity to define a group of younger pregnant women with Down syndrome risk equivalent to that of women 35 years of age and older. While low maternal serum α-fetoprotein levels are not strongly related to Down syndrome risk, approximately 20% of all cases can be identified in women under the age of 35 by the approach described here, thus offering the potential to double the number of Down syndrome cases detected while, at the same time, proportionately increasing the number of amniocenteses.

REFERENCES

1. Merkatz IR, Nitowsky HM, Macri JN, Johnson WE. An association between low maternal serum α-fetoprotein and fetal chromosomal abnormalities. AM J OBSTET GYNECOL 1984;148:886.
2. Cuckle HS, Wald NJ, Lindenbaum RH. Maternal serum alpha-fetoprotein measurement: a screening test for Down syndrome. Lancet 1984;1:926.
3. Seller MJ. Prenatal screening for Down syndrome. Lancet 1984;1:926.
4. Guibaud S, Bonnet-Capela M, Germain D, Dumont M, Thoulon JM, Berland M. Prenatal screening for Down syndrome. Lancet 1984;1:1359.
5. Tabor A, Norgaard-Pedersen B, Jacobsen JC. Low maternal serum AFP and Down syndrome. Lancet 1984; 2:161.
6. Fuhrmann W, Wendt P, Weitzer HK. Maternal serum AFP as screening test for Down syndrome. Lancet 1984;2:413.
7. Cowchock FS, Ruch DA. Low maternal serum AFP and Down syndrome. Lancet 1984;2:161.
8. Hershey DW, Crandall BF, Schroth PS. Maternal serum α-fetoprotein screening of fetal trisomies. AM J OBSTET GYNECOL 1985;153:224.
9. Spencer K, Carpenter P. Screening for Down syndrome using serum alpha-fetoprotein: a retrospective study indicating caution. Br Med J 1985;290:1940.
10. Baumgarten A, Schoenfeld M, Mahoney MJ, Greenstein RM, Saal HM. Prospective screening for Down syndrome using maternal serum AFP. Lancet 1985;1:1280.
11. Abuelo D, Bowers GB, Haddad V. Genetic counseling for low maternal serum α-fetoprotein. AM J OBSTET GYNECOL 1986;151:147.
12. Hook EB. Down syndrome, frequency in human populations and factors pertinent to variation in rates. In: de la Cruz FF, ed. Trisomy 21 research prospectives of NICHD-Mental Retardation Research Centers series. Baltimore, Maryland: University Park Press, 1981.
13. Hook EB, Cross PK, Schreinemachers DM. Chromosomal abnormality rates at amniocentesis and live-born infants. JAMA 1983;249:2034.
14. Fourth report of the UK Collaborative Study on Alpha-Fetoprotein in Relation to Neural Tube Defects. Estimating an individual's risk of having a fetus with open spina bifida and the value of repeat alpha-fetoprotein testing. J Epidemiol Community Health 1982;36:87.
15. Wald NJ, Cuckle HS, Boreham J. Alpha-fetoprotein screening for open spina bifida: effect of routine biparietal diameter to estimate gestational age. Rev Epidemiol Sante Publique 1984;32:62.
16. Baumgarten A. AFP screening and Down syndrome. Lancet 1985;1:751.
17. Palomaki GE, Knight GJ, Kloza EM, Haddow JE. Maternal weight adjustment and low serum alpha-fetoprotein values. Lancet 1985;1:468.
18. Knight GJ, Palomaki GE, Haddow JE. Maternal serum AFP: a test kit problem. N Engl J Med 1986;314:516.

Delivery of fertility control services by male and female obstetrician-gynecologists

Carol S. Weisman, Ph.D., Constance A. Nathanson, Ph.D., Martha Ann Teitelbaum, M.P.H., Ph.D., Gary A. Chase, Ph.D., and Theodore M. King, M.D., Ph.D.
Baltimore, Maryland

Sex differences in the delivery of fertility control services were explored in a national survey of 1420 recently trained obstetrician-gynecologists in active practice. Women were found to be more likely than men to provide abortion services but less likely than men to provide amniocentesis and certain infertility services. Women were found to contribute less than their proportionate share of two services for which volume was measured: artificial inseminations and sterilizations. Physician gender, however, was a less important predictor of volume of sterilizations delivered than were a set of practice-related variables. Overall our findings suggest that the increased representation of women among obstetrician-gynecologists could influence the delivery of a few specific services. (Am J Obstet Gynecol 1987;156:464-9.)

Key words: Fertility control, women physicians, sterilization

This study was undertaken to explore how the increasing number of women entering practice in obstetrics-gynecology is likely to affect the delivery of fertility control services. A previous study of residents in obstetrics-gynecology in 1981 showed that women are more likely than men to support abortion, to approve of contraceptive services to 13- or 14-year-olds without parental consent, and to be interested in health education for women.[1] A recent survey by the American College of Obstetricians and Gynecologists[2] found that women obstetrician-gynecologists were more likely than men to report that abortions are justified under some circumstances. These findings suggest that female physicians might be more likely than men to provide these services, might provide them in a more supportive or empathetic manner, or might give greater attention to the cognitive aspects of fertility control. On the other hand, some have suggested that women will provide smaller numbers of surgical procedures, as compared with men.[3] We conducted a national survey of recently trained obstetrician-gynecologists to determine whether there are discernible sex differences in the number and types of fertility control services provided to patients. In this study, fertility control services include contraceptive services, abortion services, and diagnosis and treatment of infertility. In addition, services include specific procedures as well as counseling and referrals.

Methods

Data for this analysis are from a 1984 national survey of obstetrician-gynecologists consisting of a telephone interview and a self-administered questionnaire mailed to respondents to the interview. The target population consisted of all men and women who had graduated from medical school between 1974 and 1979 (the period of greatest increase in the number of women entering the specialty), who had completed residency training in obstetrics-gynecology, and who were in active practice at the time of the survey. The sampling frame selected was the membership roster of the American College of Obstetricians and Gynecologists, since this was the most efficient way of identifying physicians formally trained in obstetrics-gynecology. To assess whether the ACOG sampling frame adequately covered the target population, we conducted a survey of a sample of 200 physicians who had completed residency programs in 1983; using last-known addresses supplied by the residency programs, we traced the physicians and telephoned them to determine their ACOG membership status and current activity status in the profession. We found that 68% were ACOG members as of 8 months after residency and that there was no sex difference in membership rates. Further, nonmembers and members did not differ significantly with regard to activity status or type of practice arrangement. We

From the Department of Behavioral Sciences and Health Education, the Department of Population Dynamics, the Department of Mental Hygiene, School of Hygiene and Public Health, and the Department of Gynecology and Obstetrics, School of Medicine, The Johns Hopkins University.
This research was funded by Grant No. R01 HD17135 from the National Institute of Child Health and Human Development.
Received for publication July 10, 1986; accepted August 19, 1986.
Reprint requests: Dr. Carol S. Weisman, Department of Behavioral Sciences and Health Education, School of Hygiene and Public Health, The Johns Hopkins University, 624 North Broadway, Baltimore, MD 21205.

Table I. Delivery of fertility control services during last year by sex*

Service	Percent who provide		Percent who refer†	
	Men	Women	Men	Women
Contraceptive counseling-referral	99.3 (n = 706)	99.4 (n = 712)	40.0 (n = 5)	75.0 (n = 4)
Prescriptions for oral contraceptives	99.4 (n = 706)	99.4 (n = 712)	50.0 (n = 4)	100.0 (n = 4)
Insertions of intrauterine contraceptive devices	93.9 (n = 706)	94.0 (n = 712)	51.2 (n = 43)	60.5 (n = 43)
Diaphragm fittings	97.5 (n = 706)	97.5 (n = 712)	27.8 (n = 18)	61.1‡ (n = 18)
Sterilization for contraceptive purposes	96.9 (n = 706)	95.9 (n = 712)	50.0 (n = 22)	69.0 (n = 29)
Abortion counseling-referral	80.3 (n = 706)	85.0‡ (n = 712)	86.3 (n = 139)	80.4 (n = 107)
First-trimester abortions	41.8 (n = 706)	48.5‡ (n = 711)	92.9 (n = 411)	92.9 (n = 366)
Second-trimester abortions	18.7 (n = 706)	21.6 (n = 712)	86.8 (n = 574)	86.9 (n = 558)
Genetic counseling	73.7 (n = 706)	69.9 (n = 712)	96.2 (n = 186)	94.4 (n = 214)
Amniocentesis	82.6 (n = 706)	70.1‡ (n = 712)	86.2 (n = 123)	89.7 (n = 213)
Infertility counseling-referral	96.7 (n = 706)	95.1 (n = 712)	78.3 (n = 23)	82.9 (n = 35)
Infertility testing	95.8 (n = 706)	92.1‡ (n = 711)	76.7 (n = 30)	80.4 (n = 56)
Infertility drugs or treatment	93.3 (n = 706)	86.7‡ (n = 712)	74.5 (n = 47)	77.9 (n = 95)
Artificial insemination	46.2 (n = 706)	34.3‡ (n = 712)	68.7 (n = 380)	64.7 (n = 303)
In vitro fertilization or implantations	2.3 (n = 706)	2.2 (n = 712)	58.4 (n = 690)	58.5 (n = 696)
Ovum transplantations	0.8 (n = 706)	0.3 (n = 711)	34.6 (n = 242)	36.2 (n = 709)

*Percent reporting that they provided (or referred for) the given service during the last year of practice; service could have been provided in any of the practice settings in which respondents worked. (Base numbers are in parentheses.)

†Among those respondents who reported that they did not provide the service.

‡χ^2 Test for the sex difference is significant, $p < 0.05$.

concluded that the ACOG sampling frame was appropriate for our purposes and did not introduce a sex bias.

The sample selected from the ACOG roster consisted of 100% of women who had graduated from medical school between 1974 and 1979 and a 28% systematic sample of comparable men: 930 men and 930 women were selected in this way. Subjects were then contacted by telephone, invited to participate in the study, and screened for eligibility on two additional criteria: completion of residency training in obstetrics-gynecology and current active practice. Fifteen subjects could not be located, and 253 refused to participate. Of the remaining subjects, 172 (9% of the original sample) were ineligible, primarily because they were still in full-time residency or postresidency training; only eight subjects (three men and five women) were "inactive" (neither in training nor in practice). There were no sex differences in reasons for ineligibility.

Eighty-four percent of the adjusted sample (original sample minus the ineligibles) completed the telephone interview (N = 1420). Among those interviewed, 84% then completed the mailed questionnaire. These are high response rates for a physician survey, and there were no sex differences in response rates to either instrument.

Respondents were located in all 50 states and had been in practice an average of 3 years at the time of our survey. The types of practice arrangements in which the respondents worked, their hours of work, and their numbers of encounters with patients have been described in a previous publication.[4]

Data reported in this paper include which fertility control services were provided by the physicians in any of the settings in which they practiced and, for selected services, the volume (number of units of the procedure) provided during the last year of the physician's practice. These data were obtained in the telephone interview, and physicians were instructed to give their best estimates for the volume figures. Self-reported volume

Table II. Comparison of observed and expected proportions of selected services provided by men and women

Service	Sample				Population estimates		
	% of Men who provide	*Mean No. provided by men**	*% of Women who provide*	*Mean No. provided by women**	*Estimated population mean†*	*Estimated total No. of procedures performed in population*	*Estimated % of services provided by women*
Sterilizations	96.9 (n = 706)	52.5 (n = 677)	95.9 (n = 712)	41.3‡ (n = 677)	50.1 (n = 3,716)	186,241	17.4§
First-trimester abortions	41.8 (n = 706)	83.9 (n = 289)	48.5 (n = 711)	68.6 (n = 341)	80.3 (n = 1,661)	133,290	20.5
Second-trimester abortions	18.7 (n = 706)	19.9 (n = 130)	21.6 (n = 712)	15.5 (n = 154)	18.9 (n = 743)	14,007	19.6
Artificial inseminations	46.2 (n = 706)	33.7 (n = 325)	34.3 (n = 712)	23.5 (n = 242)	32.0 (n = 1,678)	53,697	12.3§

*Based on number of respondents who provide the service.

†n = Estimated number of eligible members of the target population who provide the service.

‡Sex difference in means was significant ($p < 0.05$) by t test.

§The estimate differed from the reference value of 21.3% by >2 SE (see text).

data should be viewed as estimates subject to possible rounding error and recall problems. It should be noted, however, that respondents answered the questions on volume with little difficulty; fewer than 10 respondents per question declined to report volume data. Furthermore, our findings regarding percentages of physicians providing certain services and average volume of certain services (for instance, sterilizations) are quite similar to those found in a recent survey of provision of fertility control services by a sample of private office-based obstetrician-gynecologists.[5, 6] Sex differences were not investigated in that survey.

Results

More than 90% of both men and women reported that their principal professional activity was direct patient care, and only two respondents provided no direct patient care. Only one woman and two men reported that they were not providing any fertility-control services in their practices, and 23% of men and 30% of women reported that "all or most" of their patient encounters in a typical workweek involved fertility control services.

Sixteen types of fertility control services are displayed in Table I, with proportions of men and women who reported that they had "personally delivered" any of these services during the last year of their practices and, if not, that they had referred patients elsewhere for the services. Virtually all men and all women reported that they provided contraceptive counseling-referral, prescriptions for oral contraceptives, diaphragms, sterilizations for contraceptive purposes, and infertility counseling-referral. These services appear to represent a core of fertility control services provided by the physicians in this sample. The services provided by the fewest respondents (in vitro fertilizations or implantations and ovum transplants) are still relatively new technologies.

There are few substantial sex differences in Table I. Women were significantly more likely than men to provide abortion counseling-referral and first-trimester abortions. If first- and second-trimester abortions are combined, 50.5% of women and 42.9% of men provided any abortions, a small but statistically significant sex difference. (In another publication we investigated the reasons why women are more likely to provide abortions, and we found that the major reason is that women have fewer ethical reservations than men about performing abortions under a variety of circumstances.[7]) Men were significantly more likely than women to provide amniocentesis, infertility testing, infertility drugs or treatment, and artificial insemination. The largest sex difference in the table occurs for amniocentesis, which was provided by 70% of women and 83% of men. This difference is not explained by subspecialty training or by access to a hospital that provides amniocentesis.

In Table II, data are presented on the volume of selected fertility control services reported for the last year of practice. (Data on volume were obtained for six services, but the numbers of respondents providing in vitro fertilizations and ovum transplants were too small for analysis.) As shown in the left-hand side of Table II, the tendency was for men to provide more units of each service than women; however, the sex difference is significant only for sterilizations (with men providing an average of 11.2 more sterilizations during the year), due to the smaller within-sex range for sterilizations.

Comparing the sample means for men and women

Table III. Determinants of number of sterilizations performed by men and women (n = 1375)

	Mean or percentages		*Standardized partial regression coefficient**
	Men	*Women*	
Sex (female)	—	—	−0.11†
No. of years in practice	3.5	2.7‡	0.12†
No. of hours worked per week	68.7	63.2‡	0.12†
No. of patient encounters per week	150.1	129.3‡	0.23†
Type of practice			
Private solo	26%	23%	—§
Obstetrics-gynecology partnership	43%	33%‖	−0.01
Multispecialty group	14%	22%‖	0.14†
Hospital staff	10%	10%	0.06†
Academic position	8%	12%‖	−0.04
Hospital provides laparoscopy	87%	91%‖	0.07†
Urban location	52%	61%‖	−0.12†
Region			
Northeast	16%	25%‖	—§
North central	19%	19%	0.14†
South	43%	32%‖	0.13†
West	21%	24%	0.08†
R^2	—	—	0.21
Multiple R	—	—	0.45
Significance	—	—	F = 27.10, $p < 0.001$

*The dependent variable is a square-root transformation of the number of sterilizations performed during the last year of practice (range 0 to 295).

†Univariate F test significant, $p < 0.05$.

‡*t* Test for difference of means significant, $p < 0.05$.

§Omitted (reference) category.

‖χ^2 Test for sex difference significant, $p < 0.05$.

does not, however, address the question of whether women are contributing to the overall volume of fertility control services in proportion to their representation in the target population of physicians. To answer this question, we used standard formulas for estimating population totals from a stratified sample.[8] The total number of procedures performed by the target population was estimated by extrapolating from the rates found in the sample and allowing for potential ineligibility among nonsampled members of the target population. The number of procedures provided by women was then divided by the estimated total number of procedures to get the estimated percentage of services provided by women. This figure was compared, in turn, to the estimated overall proportion of women in the target population (21.3%), with the use of an ad hoc interval estimate. (The interval estimate was constructed by computing the sample variance of the total number of procedures performed by men and disregarding the negligible sampling error in women.) When the estimated proportion of services provided by women differed by >2 SE from the 21.3% that women represent of the target population, the difference was considered significant.

As demonstrated in the right-hand side of Table II, women provided a proportionate share of the total number of abortions as the result of two opposing trends: their higher likelihood of providing the service offsets their tendency to provide a somewhat lower volume of abortions. However, women provided less than their proportionate share of sterilizations and artificial inseminations. In the case of artificial insemination (the largest discrepancy), this is because women are less likely both to provide the service and to perform a higher volume; in the case of sterilizations, women and men are equally likely to provide the service, but women perform fewer procedures. [For comparison purposes, we also found that, whereas 98% of men and 97% of women delivered babies during the last year of practice, women contributed less than their proportionate share of deliveries (17.2% of all deliveries) and provided an average of 35 fewer deliveries than men during the year.]

One possible explanation for women's lower volume of service provision was the fact that, at the time of our survey, the women had been in practice an average of nearly 1 year less than the men, because the women were concentrated in the more recent medical school graduation classes. To see whether less time in practice accounted for women's lower volume of service provision, we examined the volume of the specific services by number of years in practice. We found that the only fertility control service for which volume and years in practice were linearly associated was sterilizations and

that this association held for men but not for women. Women's volume of sterilizations maintained a fairly stable pattern and was uniformly lower than that of men, regardless of time in practice. (For comparison purposes, we found that both men and women increased their volume of deliveries with more years in practice, but women provided fewer deliveries than men at each level of practice experience.)

Since sterilization is now the leading form of contraception in the United States[9] and obstetrician-gynecologists are major providers of female sterilization services,[6] the finding that female physicians provide a slightly smaller than expected proportion of sterilizations was investigated further. In a multiple regression analysis, we explored whether a number of practice-related variables on which men and women were known to differ might account for the observed sex difference in volume of sterilizations provided (Table III). The standardized partial regression coefficients may be interpreted as the effect of the given variable, after all other variables have been taken into account, and as an indication of the relative strength of this variable as a predictor. When the number of sterilizations performed was regressed on physician's gender alone, the standardized partial regression coefficient was -0.20 ($p < 0.05$), whereas the coefficient for sex when the covariables were included (Table III) was reduced to -0.11 ($p < 0.05$).

The strongest predictor of sterilization volume was the physician's number of patient encounters per week: physicians treating more patients per week provided more sterilizations during the last year. This may be contrasted with the findings in our previous analysis of abortion performance; in that analysis, neither number of encounters nor number of hours worked by the physician predicted abortion volume.[7]

Other predictors of sterilization volume that were stronger than gender are practicing primarily in a multispecialty group (in this study, both men and women who practiced primarily in multispecialty groups provided larger numbers of sterilizations than those in other practice types); practicing in the north central or southern states (both men and women practicing in the northeast and west provided fewer sterilizations than those practicing in other regions); being in practice longer; working more hours per week; and practicing in a nonurban area (outside a large city or its suburb). With the exception of their greater likelihood of practicing in multispecialty groups, women tend to be in practice situations less conducive to high-volume sterilization services.

Attitudes relevant to provision of sterilization services were also measured in the survey. While women were significantly more likely then men to be supportive of greater patient decision power in the matter of sterilization, attitudes were not found to be associated with number of sterilizations performed. Attitudes therefore could not account for the residual sex difference in sterilization volume.

Comment

The number of sex differences in delivery of fertility control services is far outnumbered by the number of similarities in the practices of men and women in this study, and the magnitude of observed sex differences is not large. However, if the patterns established by these younger physicians hold, the increasing proportion of women in obstetrics-gynecology could have some influence on delivery of a few specific services. For instance, since women were more likely than men to provide abortions, the supply of physicians providing abortions would be expected to increase as the proportion of women in practice increases. On the other hand, the increasing proportion of women would reduce the supply of providers of amniocentesis and artificial insemination. Finally, women could contribute to a slight decline in the overall volume of sterilizations. Whether this would result in unmet consumer demand for sterilizations cannot be answered in this study.

The findings with regard to sterilizations are consistent with previous studies showing that female physicians in general tend to work fewer hours per week and have fewer patient encounters than their male colleagues.[10-12] However, the magnitude of the observed sex difference in volume is not large, and this could reflect the fact that the gap between the productivity levels of men and women physicians in general has narrowed.[13, 14] In addition, a previous paper reported that sex differences in total practice hours and in number of patient encounters per week were confined to the subgroup of the sample who were married and had children; thus, to the extent that lower volume of some services is a function of overall practice volume, the lower volume of services provided by women could be a function of the childbearing stage of the life cycle and might disappear as women age.[4]

Alternatively, the lower volume for women of some procedures could be due to a tendency by other physicians to discriminate against female physicians when referring patients for such procedures or by patients to discriminate against female physicians when seeking a provider to perform a surgical procedure such as a sterilization. Since there is evidence that women's preference for a female obstetrician-gynecologist increases with experience with a female physician,[15] effects of discrimination would be expected to diminish as more patients are served by female physicians.

A limitation of the survey approach is that it focuses on self-reported practice behaviors of physicians, and no data are available on the nature of physician-patient

interactions, quality of care provided, or patient satisfaction with care received. Therefore it cannot be discerned from these data whether the increasing proportion of women will affect these other aspects of delivery of fertility control services.

REFERENCES

1. Margolis AJ, Greenwood S, Heilbron D. Survey of men and women residents entering United States obstetrics and gynecology programs in 1981. AM J OBSTET GYNECOL 1983;146:541.
2. ACOG poll. Ob-Gyns' support for abortion unchanged since 1971. Fam Plann Perspect 1985;17:275.
3. Domenighetti G, Luraschi P, Marazzi A. Hysterectomy and sex of the gynecologist. N Engl J Med 1985;313:1482.
4. Weisman CS, Teitelbaum MA, Nathanson CA, Chase GA, King TM, Levine DM. Sex differences in the practice patterns of recently trained obstetrician-gynecologists. Obstet Gynecol 1986;67:776.
5. Orr MT, Forrest JD. The availability of reproductive health services from U.S. private physicians. Fam Plann Perspect 1985;17:63.
6. Orr MT, Forrest JD, Johnson JH, Tolman DL. The provision of sterilization services by private physicians. Fam Plann Perspect 1985;17:216.
7. Weisman CS, Nathanson CA, Teitelbaum MA, Chase GA, King TM. Abortion attitudes and performance among male and female obstetrician-gynecologists. Fam Plann Perspect 1986;18:67.
8. Hansen MH, Hurwitz WN, Madow WG. Sample survey methods and theory. Methods and applications, vol 1. New York: Wiley, 1953:122.
9. Bachrach CA, Mosher WD. Use of contraception in the United States, 1982. In: Advance data from vital and health statistics. Hyattsville, Maryland: National Center for Health Statistics, 1984; DHHS publication no. (PHS) 85-1250. (Vital and health statistics; no. 102).
10. Butter I, Carpenter E, Kay B, Simmons R. Sex and status hierarchies in the health workforce. Washington DC: American Public Health Association, 1985:25.
11. Mitchell JB. Why do women physicians work fewer hours than men physicians? Inquiry 1984;21:361.
12. American Medical Association, Center for Health Policy Research. Differences in practice characteristics between female and male physicians. Socioeconomic Monitoring System 3, Chicago, Illinois: American Medical Association, 1984.
13. Heins M, Smock S, Martindale L, Jacobs J, Stein M. Comparison of the productivity of women and men physicians. JAMA 1977;237:2514.
14. Bobula JD. Work patterns, practice characteristics, and incomes of male and female physicians. J Med Educ 1980;55:826.
15. Weisman CS, Teitelbaum MA. Physician gender and the physician-patient relationship: recent evidence and relevant questions. Soc Sci Med 1985;20:1119.

Three-contrasts method: An ultrasound technique for monitoring transcervical operations

Bao-Liang Lin, M.D., Yoshiyuki Iwata, M.D., Naohiko Miyamoto, M.D., and Shigeru Hayashi, M.D.

Kawasaki, Japan

The "three-contrasts method" of ultrasonography is designed to monitor transcervical operations for submucous uterine myomas and uterine septa when a pediatric urologic resectoscope is used. This monitoring method has proved more effective than concomitant laparoscopy. The pediatric resectoscope can also be used efficiently for diagnostic and therapeutic purposes without dilating the cervical canal. (AM J OBSTET GYNECOL 1987;156:469-72.)

Key words: Hysteroscopy, ultrasound, ultrasonography

It has previously been reported that transcervical resection of submucous uterine myomas and uterine septa can be performed by use of a resectoscope or an operating hysteroscope. To prevent uterine perforation, these operations have always been monitored by concomitant laparoscopy. When laparoscopy is being used, it is difficult to take precautions against perforation of the posterior uterine wall. In this paper we report the results of a new "three-contrasts method" of ultrasonography to monitor the transcervical resection of submucous uterine myomas and uterine septa. The three contrasts are derived from ultrasonographic examination of (1) a full urinary bladder, (2) fluid in the cul-de-sac, and (3) fluid in the uterine cavity or the inserted resectoscope. With this method a clear outline of the uterine wall and submucous myoma can be dis-

From the Department of Obstetrics and Gynecology, Kawasaki Municipal Hospital.
Received for publication September 20, 1985; revised July 28, 1986; accepted August 22, 1986.
Reprint requests: Dr. Bao-Liang Lin, Department of Obstetrics and Gynecology, Kawasaki Municipal Hospital, 12-1, Shinkawadori, Kawasakiku, Kawasaki, Kanagawaken, Japan.

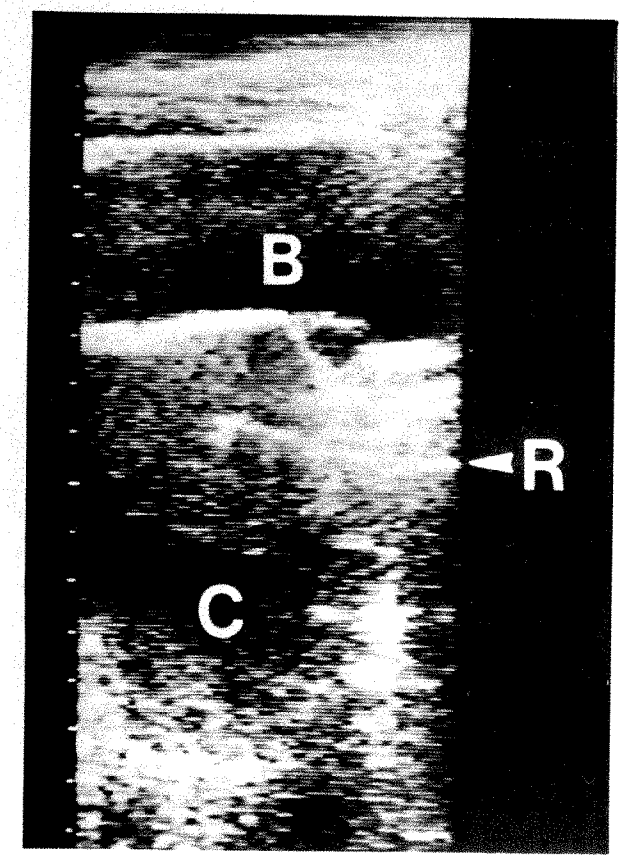


Fig. 1. Longitudinal section of the uterus after urinary bladder and cul-de-sac were filled with fluid in a case of uterine septum. The movement of the cutting loop can be seen clearly on ultrasound. *B* = Urinary bladder; *C* = cul-de-sac; *R* = resectoscope.

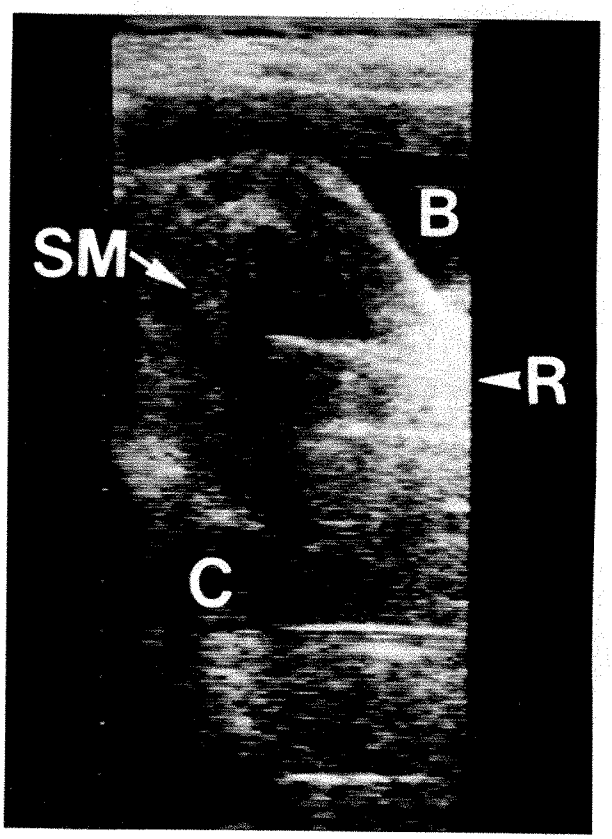


Fig. 2. Longitudinal section showing the resectoscope in the distended uterine cavity and the submucous myoma in front of the tip of the scope. *B* = Urinary bladder; *C* = cul-de-sac; *R* = resectoscope; *SM* = submucous myoma.

cerned, and injury to the uterine wall, resulting from overcutting of the scope, is prevented.

Material and methods

With the use of hysteroscopy six patients with complaints of menorrhagia and metrorrhagia were diagnosed as having submucous uterine myoma. Five were pedunculate in type and one was sessile. Menorrhagia was so severe in these patients that they all needed to use more than eight pads or tampons per day. Hemoglobin counts before iron therapy ranged from 4.7 to 10.6 gm/dl. Two other patients who complained of sterility were diagnosed (by means of hysterosalpingogram, hysteroscopy, and laparoscopy) as having uterine septa. All eight of these patients underwent transcervical operation with the use of a pediatric urologic resectoscope and ultrasound monitoring. Patients chosen for transcervical removal of submucous uterine myomas were selected according to the following criteria: (1) severe menorrhagia and/or intractable metrorrhagia, (2) uterus smaller than 9 weeks' gestational size, (3) submucous uterine myoma ≤30 mm in diameter, (4) no existence or suspicion of malignancy or premalignancy of the uterus. Diagnostic hysteroscopy was performed at our clinic with the use of a hysteroscope with an external sheath 4 mm in diameter (Olympus Optical Co.) without anesthesia or cervical dilation. After the diagnosis, all the patients underwent operative evaluation consisting of electrocardiogram, chest x-ray film, and evaluation of liver and renal function. The patient was admitted for transcervical operation when the menstrual period was over.

The night before operation, a balloon catheter was inserted in the cervical canal; the catheter was removed the next morning to facilitate insertion of the resectoscope. A completely cleansing enema was given before operation. All transcervical resection procedures were performed with the patient under general anesthesia. An intravenous indwelling catheter was inserted through the umbilicus into the abdominal cavity. This was connected, through an intravenous infusion set, to a 500 ml bottle of lactated Ringer's solution. Intra-abdominal infusion was then started. The patient was placed in Fowler's position to allow the infused fluid to accumulate in the cul-de-sac. At the same time about 200 ml of normal saline solution was introduced into

the urinary bladder through a balloon catheter. The catheter was retained throughout the operation in order to adjust the amount of fluid in the urinary bladder. An assistant then placed the transducer of a sonograph (SAL-35A, Toshiba Medical Systems Co.) in a longitudinal position in order to obtain a clear outline of the uterus, which was derived by checking the amounts of infused fluid in the cul-de-sac and urinary bladder.

A pediatric urologic resectoscope with an external sheath 4.5 mm in diameter (Olympus Optical Co.) was inserted into the uterine cavity through the cervical canal to reevaluate the status of submucous uterine myoma and uterine septum. Simultaneously, by moving the resectoscope in the distended uterine wall, the anatomic correlation of the uterine wall, submucous uterine myoma, uterine septum, and loop of the resectoscope was confirmed by means of ultrasonography (Figs. 1 and 2). The loop of the resectoscope, guided by the ultrasound, is set in the correct position for the cutting and coagulation process.

Cutting procedures were performed as follows: The loop of the resectoscope was placed on the farther side of the target and was drawn toward the objective lens of the scope. We tried to avoid shaving off a large mass while moving the cutting loop. The coagulation current was applied whenever hemostasis was required. When the cutting process was completed and the cutting site reached the normal uterine wall, the position of the resectoscope was carefully checked with ultrasound. High-frequency currents for cutting and coagulation should be turned to the lowest possible setting of diathermy in order to avoid unnecessary burn injuries. At the end of operation, the cul-de-sac was punctured, with ultrasound guidance, to remove the intra-abdominal fluid. The urinary bladder was subsequently emptied. The irrigating fluid used in the operation was a solution of either 10% pentose or 10% dextrose in water. Serum electrolytes were checked routinely before and after the operation. About 3 months after operation, hysteroscopy was performed in each patient to confirm the regeneration of the mucosa over the resected area.

Results

Resection of submucous uterine myoma of six patients, and resection of uterine septum of two patients, were performed according to the three-contrasts method described above. Each resection was done within an hour. There were no traumatic complications. No patient required laparotomy during or after operation. Blood transfusions were not required in any case. Postoperative serum electrolytes revealed no significant change in any patient. All patients were discharged within 3 days.

Clinical follow-up on the six patients with submucous uterine myoma showed improvement of metrorrhagia and menorrhagia by measuring the number of pads or tampons used during menstruation and by examining the passage of blood clots (Table I). Postoperative hysteroscopic examinations revealed no intrauterine adhesion and that regeneration of endometrium at the operation site was normal. The results of resection of uterine septum on the other two patients are still being determined by follow-up studies.

Table I. Number of pads or tampons used per day during menstruation in patients with submucous myoma

Patient No.	*No. of pads or tampons per day*	
	Before operation	*After operation*
1	13	6
2	16	2
3	20	4
4	11	1
5	21	8
6	16	6

Comment

Submucous uterine myoma, regardless of size, is one of the most annoying problems in clinical gynecology. It occasionally causes menorrhagia and metrorrhagia. Uterine septum is a cause of habitual abortion. Transcervical removal of nonmalignant intrauterine neoplasms and uterine septa has been reported elsewhere,[1-3] but operation performed by resectoscope or operating hysteroscope often carries a risk of accidental trauma, such as perforation of the uterine wall. To avoid such trauma during transcervical operations, concomitant laparoscopy has been used.[1-3] Even with concomitant laparoscopy, however, traumatic injury to the posterior uterine wall can be overlooked. Real-time ultrasound has also been used during gynecologic operations.[4] In nonpregnant patients with submucous uterine myomas, it is difficult to use ultrasound to ascertain the size of a fibroid when only the full bladder is used for contrast. It is also difficult to distinguish the posterior uterine wall from the surrounding structures. The three-contrasts method of ultrasonography may be used to overcome these problems.

Adding fluid in the cul-de-sac with a percutaneous intraperitoneal infusion provides a clear contrast to the posterior uterine wall and also mobilizes the intestine away from the uterus. Thus accidental injury to the uterus can be avoided. To obtain a clear picture when ultrasonography is used, the amount of fluid in the urinary bladder and the pressure of the transducer on the abdominal wall should be adjusted. Too much bladder volume or excessive pressure of the transducer

against the abdominal wall will reduce the amount of fluid in the cul-de-sac and diminish the discernible contrast.

Cooperation between the operating surgeon and the ultrasound operator is essential. The ultrasound operator should constantly inform the operating surgeon of the position of the resectoscope in order to avoid any accidental injury to the uterine wall. Both preoperative and postoperative ultrasonograms or hysterosalpingograms should be obtained to assure complete resection of myomas.

The advantage of using the pediatric resectoscope is that accurate observations of the intrauterine condition can be made without a need for cervical dilation. This procedure is difficult to perform with an adult-type resectoscope. Dilation of the cervical canal may cause uterine bleeding and blood clotting; it can also cause subsequent uterine contraction, which will interrupt operation. The three-contrasts method of ultrasonography makes it possible to monitor the posterior uterine wall, the submucous myoma, and the scope and thus enhances the safety of transcervical operations.

REFERENCES

1. Neuwirth RS. A new technique for and additional experience with hysteroscopic resection of submucous fibroids. Am J Obstet Gynecol 1978;131:91-4.
2. DeCherney A, Polan ML. Hysteroscopic management of intrauterine lesions and intractable uterine bleeding. Obstet Gynecol 1983;61:392-7.
3. Daly DC, Walters CA, Soto-Albors CE, Riddick DH. Hysteroscopic metroplasty: surgical technique and obstetric outcome. Fertil Steril 1983;39:623-8.
4. Romero R, Copel JA, Jeanty P, Reece EA, Reiss R, Hobbins JC. Sonographic monitoring to guide the performance of postabortal uterine curettage. Am J Obstet Gynecol 1985;151:51-3.

Prenatal ultrasonic diagnosis of multiple bowel atresias

M. Amanda Skoll, M.D., Gerald P. Marquette, M.D., and Emily F. Hamilton, M.D.
Montreal, Quebec, Canada

This case illustrates the course of an obstructive process through spontaneous perforation resulting in decompression of dilated bowel loops. The resulting meconium peritonitis appeared as fetal ascites. Postnatal investigations and laparotomy confirmed the diagnosis of a rare form of multiple congenital atresias of the bowel. (Am J Obstet Gynecol 1987;156:472-3.)

Key words: Prenatal diagnosis, bowel atresia, ascites, ultrasound

In this report, we describe a case of multiple atresias involving segments of the fetal gastrointestinal tract from stomach to rectum, with spontaneous perforation and subsequent meconium peritonitis.

Case report

Mrs. P. S., a 19-year-old primigravid woman, underwent ultrasound examination at 21 weeks' gestation that revealed multiple large fluid-filled loops of dilated bowel occupying the upper anterior region of the fetal abdominal cavity (Fig. 1). The amniotic fluid volume was normal and no other structural abnormalities were found.

Follow-up scanning from 25 to 28 weeks' gestation

From the Department of Obstetrics and Gynecology, Royal Victoria Hospital and McGill University.
Received for publication March 5, 1986; revised June 12, 1986; accepted August 12, 1986.
Reprint requests: Dr. Gerald P. Marquette, Royal Victoria Hospital, Women's Pavilion, F4.29, 687 Pine Ave. West, Montreal, Quebec, Canada H3A 1A1.

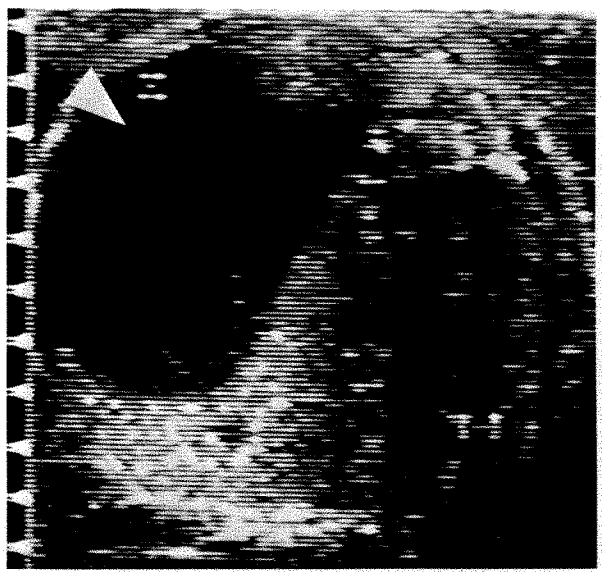


Fig. 1. Sonogram at 21 weeks' gestation. Dilated loop of bowel *(arrowhead)* in the upper part of the abdomen.

showed a reduction in the bowel distention and the appearance of fetal ascites (Fig. 2). However, from 28 to 33 weeks, an increase in dilatation became apparent and was accompanied by gradual disappearance of the ascites. Concurrent with resolution of the ascitic fluid, a calcified collection became evident in the right lower quadrant and remained constant.

At 35⅚ weeks' gestation, the patient was delivered spontaneously of a 2400 gm male infant. The baby had significant abdominal distention. Subsequent laparotomy revealed numerous sites of atresia throughout the bowel. Beginning in the stomach, multiple septa divided the intestine into segments of 2 to 3 cm, and the calcified deposits that had been previously noted on ultrasound were entirely within the lumen.

Comment

The presence of multiple distended loops of bowel is compatible with a distal obstructive process. Fetal ascites in combination with other signs of bowel obstruction suggests the occurrence of spontaneous perforation with subsequent meconium peritonitis.[1] Most commonly, meconium peritonitis results from obstruction secondary to meconium ileus or low atresia. Intraperitoneal calcifications have been described as a sequela of meconium peritonitis, due to the precipitation of tissue calcium by the fatty components of meconium.[1]

In our case, the initial picture was compatible with proximal intestinal obstruction. However, the subsequent appearance of ascites seemed to represent meconium peritonitis, more in keeping with low obstruction. Findings at laparotomy explained the ultrasound results by confirming both upper and lower bowel atresias. This rare condition[2] is associated with pasty calcification within the bowel lumen.



Fig. 2. Sonogram at 25 weeks' gestation. Decreased dilatation of bowel loops with presence of ascites *(arrowhead)*. Same level as Fig. 1.

This is the first known reported case of multiple atresias of this type described on ultrasonography. It demonstrates that intra-abdominal calcifications may not always represent intraperitoneal deposits. It also provides an example of the features of simultaneous upper and lower intestinal obstruction.

REFERENCES

1. Williams J, Nathan RD, Worthen NJ. Sonographic demonstration of the progression of meconium peritonitis. Obstet Gynecol 1976;47:618.
2. Guttmann FM, Braun P, Garance PH, et al. Multiple atresias and a new syndrome of hereditary multiple atresias involving the gastrointestinal tract from stomach to rectum. J Pediatr Surg 1973;8:633.

Effect of naloxone on fetal behavior near term

Domenico Arduini, M.D., Giuseppe Rizzo, M.D., Sergio Dell'Acqua, M.D., Salvatore Mancuso, M.D., and Carlo Romanini, M.D.

Rome and Ancona, Italy

Fetal behavior was studied after intravenous administration of either 0.4 mg of naloxone or an equal volume of saline solution in 54 healthy pregnant women near term. The number, duration, and amplitude of fetal heart rate accelerations increased after naloxone injection. The incidence of both gross fetal body movements and fetal breathing movements increased, especially in the first hour after naloxone administration. The distribution of fetal behavioral states was modified with a prevalence of active sleep and active awake states compared to the quiet sleep state. These data suggest that endorphins could be involved in the modulation of fetal behavior. (Am J Obstet Gynecol 1987;156:474-8.)

Key words: Naloxone, fetal heart rate, fetal behavior

Within the past few years several authors have shown the existence of ultradian and circadian rhythms of various fetal biophysical activities such as gross fetal body movements, fetal breathing movements, fetal eye movements, and fetal heart rate patterns.[1-3] The association of these activities and the increased understanding of fetal physiology have permitted the detection near term of behavioral states similar to those in the newborn infant.[4]

There is no account, however, of the mechanism regulating the alternation of these states. Recently it was suggested that the fluctuations of endorphin levels could be involved in the regulation of sleep states in adults.[5]

We investigated the effects of naloxone on echographic and cardiotocographic parameters to determine whether fetal endorphin levels are related to fetal behavior.

Material and methods

On the basis of several reports on the absence of detrimental effects of naloxone on healthy fetuses,[6, 7] the ethical committee of our institution approved this investigation. After informed consent had been obtained, the fetuses of 54 healthy pregnant women with gestational ages between 37 and 39 weeks were studied.

Studies were conducted in the afternoon, 2 hours after a standardized 1000 kcal lunch. Recordings were performed on patients lying in left semirecumbent position in a quiet room. They underwent simultaneous cardiotocographic and echographic examination.

Aloka SSD 248 and Aloka SSD 210 SX real-time scanners with 3.5 MHz multifocused linear transducers were used for examination of fetal head and trunk, respectively. Permanent records of all the images were made for later analysis on beta format videocassettes with use of Sony video recorders (Models SL HF 100 EC).

With use of a technique previously reported,[3] three parameters were evaluated: gross fetal body movements, fetal eye movements, and fetal breathing movements. These movements were simply classified as present or absent, without further differentiation as to the type of movement. Fetal heart rate was obtained by means of abdominal electrocardiography with a Hewlett-Packard 8030A cardiotocograph.

For the purpose of this investigation we identified fetal heart rate accelerations as increases of at least 15

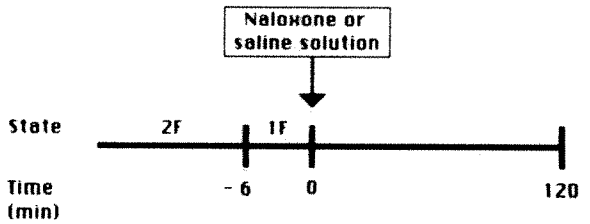


Fig. 1. Experimental design. State *1F* = quiet sleep and *2F* = active sleep.

Table I. Criteria for classification of fetal behavioral states[5]

Behavioral state	*Fetal heart rate pattern*	*Fetal eye movement*	*Fetal body movement*
Quiet sleep (1 F)	A	Absent	Absent†
Active sleep (2 F)	B	Present	Periodic
Quiet awake (3 F)	C	Present	Absent
Active awake (4 F)	D	Present	Continuous

*For definitions of patterns see text.

†Absent or incidental.

From the Departments of Obstetrics and Gynecology, Universita' Cattolica S. Cuore Roma and Universita' di Ancona.

Received for publication December 10, 1985; revised August 21, 1986; accepted August 27, 1986.

Reprint requests: Dr. Domenico Arduini, 1st Clinica Ostetrica e Ginecologica, Universita' Cattolica S. Cuore, Largo A. Gemelli, 8, 00168 Roma, Italy.

Table II. Characteristics of the two groups of patients

	Group A (naloxone)	*Group B (control)*	*F tests**		t *test*	
			Value	*P*	*Value*	*P*
Age (yr)	26.3 ± 3.1	27.9 ± 2.7	1.3	NS	2.0	NS
Parity (no)	1.6 ± 1.4	1.7 ± 1.2	1.3	NS	0.5	NS
Gestational age (wk)	38.1 ± 0.7	37.9 ± 0.9	0.6	NS	1.3	NS
Birth weight (gm)	3525 ± 419	3434 ± 516	0.6	NS	0.7	NS
Apgar score, 1 min	>7	>7	–	–	–	–
Apgar score, 5 min	>8	>8	–	–	–	–

*No differences were found between the variances as demonstrated by the F test. Significance was therefore evaluated by means of unpaired *t* test with variance assumed to be equal.

Table III. Characteristics of cardiotocographic and echographic parameters during the 30 minutes preceding the intravenous injection

	Group A (naloxone)	*Group B (control)*	*F test**		t *test**	
			Value	*P*	*Value*	*P*
Accelerations (no)	14.8 ± 3.9	15.2 ± 4.1	0.9	NS	0.3	NS
Amplitude (bpm)	20.4 ± 4.4	20.8 ± 4.3	1.0	NS	0.3	NS
Duration (sec)	44.7 ± 7.6	42.5 ± 7.8	0.9	NS	1.0	NS
Fetal body movement (n)	13.3 ± 3.8	13.9 ± 4.0	0.9	NS	0.5	NS
Time spent breathing (%)	20.8 ± 11.3	19.8 ± 9.7	1.3	NS	0.3	NS

*No differences were found between the variances as demonstrated by the F test. Significance was therefore evaluated by means of unpaired *t* test with variance assumed to be equal.

Table IV. Characteristics of fetal heart rate accelerations

	Group A (naloxone)	*Group B (control)*	*F test**		t *test**	
			Value	*P*	*Value*	*P*
Accelerations						
1st hr	47.1 ± 8.6	30.6 ± 6.4	1.8	NS	7.9	0.001
2nd hr	36.4 ± 7.1	31.4 ± 6.6	1.1	NS	2.6	0.02
Amplitude (bpm)						
1st hr	29.4 ± 5.3	21.6 ± 4.1	1.6	NS	6.0	0.001
2nd hr	24.3 ± 4.9	20.9 ± 4.2	1.3	NS	2.7	0.01
Duration (sec)						
1st hr	56.8 ± 10.1	42.1 ± 7.8	1.6	NS	5.9	0.001
2nd hr	49.4 ± 8.7	43.1 ± 8.3	1.0	NS	2.7	0.01

*No differences were found between the variances as demonstrated by the F test. Significance was therefore evaluated by means of unpaired *t* test with variance assumed to be equal.

bpm for 15 seconds. Maximum amplitude and duration of acceleration were also measured.

According to Nijhuis et al.,[4] by means of a 3-minute moving window, four different patterns of fetal heart rate could be recognized: (A) heart rate stable with a variability of <10 bpm and isolated accelerations strictly related to movements; (B) heart rate with a variability >10 bpm and presence of numerous accelerations; (C) heart rate stable with a variability of >10 bpm and absence of accelerations; (D) heart rate unstable with large and long-lasting accelerations.

The association between fetal heart rate pattern, fetal body movement, and fetal eye movement makes it possible to detect four different behavioral states (quiet sleep, active sleep, quiet awake, and active awake) as reported in Table I. The simultaneous agreement of these three parameters was considered necessary for classification into one of these four states. In the absence of this condition epochs were classified as periods of no coincidence.

Fetal breathing movements were analyzed independently in consecutive 1-minute periods. The proportion of each consecutive 1-minute period during which fetal breathing movements were present was analyzed to evaluate its respective incidence (% time spent breathing). Moreover, the number of fetal body movements during the recordings was analyzed.

When two consecutive 3-minute quiet sleep epochs immediately preceded by the state of active sleep were identified, a person other than the investigators intra-

Table V. Characteristics of fetal body movement and fetal breathing movement

	Group A (naloxone)	*Group B (control)*	*F test**		*t test**	
			Score	*P*	*Score*	*P*
Fetal body movement (n)						
1st hr	46.9 ± 7.8	29.7 ± 6.8	1.3	NS	8.6	0.001
2nd hr	35.8 ± 6.9	30.3 ± 6.7	1.0	NS	2.9	0.01
Time spent breathing (%)						
1st hr	64.8 ± 10.9	19.6 ± 9.6	1.2	NS	16.1	0.001
2nd hr	48.9 ± 12.3	23.2 ± 11.1	1.2	NS	8.0	0.001

*No differences were found between the variances as demonstrated by the F test. Significance was therefore evaluated by means of unpaired *t* test with variance assumed to be equal.

Table VI. Distribution of fetal behavioral states (3-minute periods) after intravenous injection

	Quiet sleep		*Active sleep*		*Quiet awake*		*Active awake*		*No coincidence*	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
Group A	113	11.4	702	70.8	23	2.8	85	8.6	69	6.9
Group B	246	24.5	647	64.4	26	2.5	42	4.1	45	4.5

$\chi^2 = 71.2$; $p < 0.001$.

Table VII. Time interval and distribution of the changes of state after intravenous injection

	Lag-time (min)	*Active sleep*		*Quiet awake*		*Active awake*	
		n	%	*n*	%	*n*	%
Group A	9.6 ± 2.3*	15	55.5	0	0	12	44.5
Group B	16.4 ± 7.2*	23	85.1	3	11.1	1	3.8

$\chi = 13.9$; $p < 0.001$.
*F test ($F = 9.8$; $p < 0.001$). Unpaired *t* test with variance assumed to be unequal ($t = 4.7$; $p < 0.001$).

venously administered either 0.4 mg of naloxone hydrochloride (Narcan, Crinos, Como Villaguardia, Italy) or an equal volume of saline solution (0.9% sodium chloride). Observers involved in this investigation were not informed of the solution injected. The choice to administer either naloxone or placebo was randomized. Thus two groups were arranged: group A received naloxone (n = 27) and group B received a placebo (n = 27). Recordings were then continued for a further 2 hours. The experimental design is reported in Fig. 1.

Differences in fetal behavior between the two groups of patients were analyzed. All data are expressed as mean values ± standard deviations. Statistical analysis was performed by means of the χ^2 test and unpaired *t* test. Equality of variance was always determined by means of the F test.

Results

The overall characteristics of the two groups of patients are given in Table II. No differences were found between the two groups. Normal fetal outcomes confirmed the good health of the fetuses studied.

The intervals between the beginning of recording and naloxone or placebo administration were similar in the two groups (group A, 46.9 ± 12.3 minutes; group B, 49.3 ± 14.2 minutes). No differences were found in the echographic and cardiotocographic parameters preceding the intravenous infusion as shown in Table III.

The characteristics of fetal heart rate accelerations are shown in Table IV.

An increase in the number of accelerations in group 1 was especially evident in the first hour after treatment. Similar trends were found for the amplitude and duration of accelerations.

Table V gives the number of fetal body movements and the percent of time spent breathing. Both fetal body movements and fetal breathing movements increased after naloxone administration, especially during the first hour. The number of fetal body movements (Fig. 2) and the percent of time spent breathing (Fig. 3) are plotted as grouped means and standard deviations for each 10-minute interval after intravenous injection.

The percent of time in which a good view of fetal

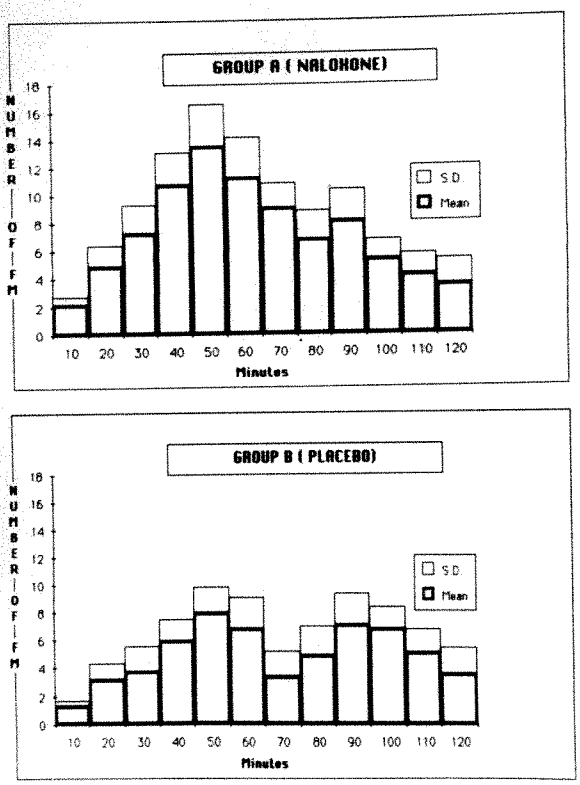


Fig. 2. The incidence of fetal body movements after endovenous injection of either naloxone (group A) or saline solution (group B).

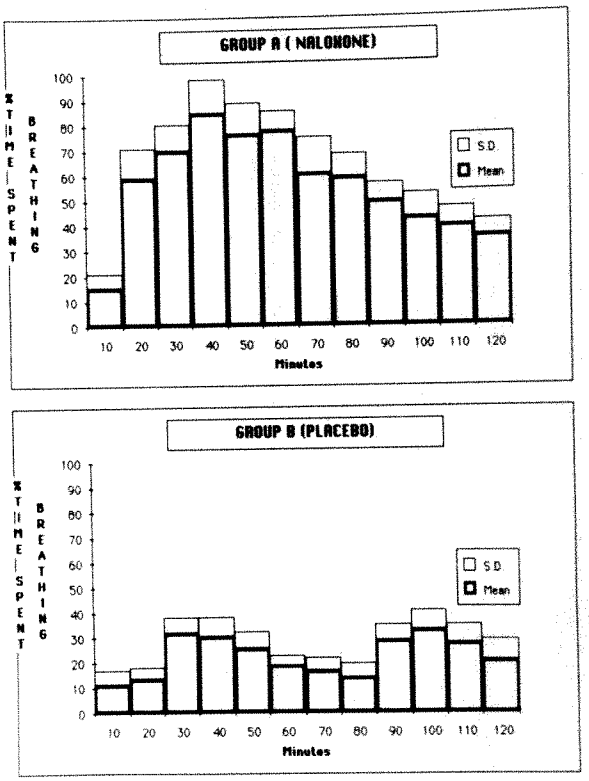


Fig. 3. Percent of time spent breathing after endovenous injection of either naloxone (group A) or saline solution (group B).

lens and trunk was obtained simultaneously with a reliable recording of fetal heart rate was similar in the two groups (group A, 91.9%; group B, 93.2%).

The distribution of fetal behavioral states is given in Table VI. We found an increase in the incidence of active sleep and active awake states and a decrease of the quiet sleep state in group 1 in comparison to the control group. No differences were found for the quiet awake state.

The lag-time between the intravenous injection and the change of state was measured. In group A, the quiet sleep state changed after 9.6 ± 2.3 minutes, while in group B (control) it changed after 16.4 ± 7.2 minutes. The F test demonstrated the difference between the variances of the lag-time ($p < 0.001$). An unpaired t test with variances assumed to be unequal was performed; the difference between the two groups is statistically significant ($p < 0.001$).

As shown in Table VII, in group A the quiet sleep state was followed in 15 cases (55.5%) by the active sleep state and in 12 (44.5%) by the active awake state. In group B the quiet sleep state was followed by the active sleep state in 23 cases (85.1%), quiet awake in three cases (11.1%), and active awake in one case (3.8%). This distribution is statistically significant (χ^2; $p < 0.001$).

Comment

There are few data on the effects of naloxone on fetal behavior. Goodlin[8] showed that flat fetal heart rate returned to a normal variability 20 minutes after intramuscular naloxone injection. Umans and Szeto[9] demonstrated in narcotic addict fetal lambs an increased number of fetal body movements, fetal eye movements, and fetal breathing movements after naloxone administration. Adamson et al.[10] found that the administration of naloxone increased the incidence of fetal breathing movements in the fetal lamb.

Until now, however, no data were available on the effects of naloxone on the behavior of the healthy human fetus. Our results show an increase in the number of fetal heart rate accelerations, fetal body movements, and fetal breathing movements, within 10 minutes of intravenous naloxone administration. Moreover, we found a modification in the distribution of fetal behavioral states with a prevalence of the active sleep and active awake states.

The mechanism regulating fetal behavior is still unknown. Nathanielsz et al.[11] found a relationship between the alternation of fetal behavior and uterine contractures in the pregnant sheep. The mechanisms involved may include small falls in the level of fetal oxygenation or compression of the fetal body. Hofmeyr

et al.,[12] however, recently demonstrated that the uterine contractions are rarely found in association with the rhythms of fetal behavior.

Terao et al.[13] found a biphasic pattern of heart rate in anencephalic fetuses with traces of cerebral cortex and normal or hypoplastic midbrain. Moreover, the aplasia of midbrain is characterized by the loss of biphasic pattern and the reduction in the number of accelerations. Thus the occurrence of accelerations and ultradian rhythms of fetal heart rate seems to be related to the presence of midbrain.

We have previously suggested that maternal plasma cortisol could be involved in the regulation of fetal behavior through the modulation of fetal pituitary gland.[14] Preliminary findings[15] support this hypothesis: the inhibition of the fetal pituitary-adrenal axis, after triamcinolone administration, causes modifications of fetal behavior similar to those found in this investigation.

The mechanism of action could be the same. The pituitary gland secretes adrenocorticotropic hormone and β-endorphin concomitantly, since both are derived from a common precursor, namely, pro-opiomelanocortin.[16] Therefore triamcinolone suppresses the secretion of both adrenocorticotropic hormone and β-endorphin, whereas naloxone antagonizes the action of β-endorphin.

Further studies are required for a complete understanding of the mechanisms regulating fetal behavior, but these data suggest that endorphins could be involved in the modulation of fetal behavior.

REFERENCES

1. Patrick J, Campbell K, Carmichael L, Natale R, Richardson B. Patterns of fetal body gross movements over 24-hour intervals during the last 10 weeks of pregnancy. Am J Obstet Gynecol 1982;142:363.
2. Patrick J, Campbell K, Carmichael L, Natale R, Richardson B. Patterns of human fetal breathing movements during the last 10 weeks of pregnancy. Obstet Gynecol 1980;56:24.
3. Arduini D, Rizzo G, Giorlandino C, et al. The fetal behavioural states: an ultrasonic study. Prenat Diagn 1985; 5:296.
4. Nijhuis JG, Prechtl HFR, Martin CB Jr, Bots RSGM. Are there behavioral states in the human fetus? Early Hum Dev 1982;6:177.
5. Mullen PE. Sleep and its interaction with endocrine rhythms. Br J Psychiatry 1983;142:215.
6. Payne JP. Narcotic antagonists and their use in obstetrics. Br J Anaesth 1973;45:794.
7. Chang A, Gilbert M, Wood C, Humphrey M. The effects of nalorphine and naloxone on maternal and fetal blood gas and pH. Med J Aust 1976;1:263.
8. Goodlin RC. Naloxone and its possible relationship to fetal endorphin levels and fetal distress. Am J Obstet Gynecol 1981;139:16.
9. Umans JG, Szeto HH. Precipitated opiate abstinence in utero. Am J Obstet Gynecol 1985;151:441.
10. Adamson SL, Patrick JE, Challis JRG. Effects of naloxone on the breathing, electrocortical heart rate, glucose and cortisol responses to hypoxia in the sheep fetus. J Dev Physiol 1984;6:485.
11. Nathanielsz PW, Bailey A, Poore ER, Thorburn GD, Harding R. The relationship between myometrial activity and sleep state and breathing in fetal sheep during the last third of pregnancy. Am J Obstet Gynecol 1980;138:653.
12. Hofmeyr GJ, Bamford OS, Gianapoulus JG, Parkes MJ, Dawes GS. The partial association of uterine contractions with changes in electrocortical activity, breathign and Pa_{O_2} in the fetal lamb: effects of brain stem section. Am J Obstet Gynecol 1985;152:905.
13. Terao T, Kawashima Y, Noto H, et al. Neurological control of fetal heart rate in 20 cases of anencephalic fetuses. Am J Obstet Gynecol 1984;149:201.
14. Arduini D, Rizzo G, Parlati E, Dell'Acqua S, Mancuso S, Romanini C. Are the fetal heart rate patterns related to fetal-maternal endocrine rhythms at term of pregnancy? J Foetal Med (in press).
15. Arduini D, Rizzo G, Parlati E, et al. Modifications of ultradian and circadian rhythms of fetal heart rate after fetal-maternal adrenal gland suppression: a double-blind study. Prenat Diagn 1986;6:409.
16. Mains RE, Eipper BA, Ling N. Common precursors to corticotropins and endorphins. Proc Natl Acad Sci USA 1977;74:3014.

Immunocytochemistry of human chorionic gonadotropin in human chorionic villi

Kunshige Hamasaki, M.D., Yasushi Okamura, M.D., Hiroshi Ueda, Ph.D., Hirohiko Kagawa, Ph.D., and Sunao Fujimoto, M.D.

Kitakyushu, Japan

The immunocytochemical localization of human chorionic gonadotropin was investigated in chorionic villi from the seventh to twelfth week of gestation. By the light microscopic peroxidase-antiperoxidase tecnhique, positive reactions of human chorionic gonadotropin were found exclusively in the syncytiotrophoblast. Immunoelectron microscopy by means of the protein A–gold technique reveals localization of the immunoreactive gold particles in two kinds of membrane-bound granular inclusions in this cell; one type is granules of 200 to 300 nm in diameter with moderate electron density and the other is large electron-dense bodies of 500 to 1000 nm. The former seems to be Golgi-derived secretory granules that play a role in the release of human chorionic gonadotropin from the syncytiotrophoblast. Although the origin of the latter is still uncertain, a certain amount of this hormone might be stored or treated by lysosomal digestion in the large bodies during these stages. (Am J Obstet Gynecol 1987;156:479-83.)

Key words: Human chorionic villi, syncytiotrophoblast, human chorionic gonadotropin, immunoelectron microscopy

The localization of human chorionic gonadotropin in human placentas has been studied by several investigators. An earlier worker[1] considered cytotrophoblast (Langhans' cell) as a human chorionic gonadotropin–producing cell on the basis of the correlation between the hormonal assay and the cell number.[1] With the light microscopic immunofluorescence and immunoperoxidase techniques together with the specific antisera against human chorionic gonadotropin, localization of this hormone has been demonstrated in the syncytiotrophoblast[2] and in both the trophoblastic layers.[3] Furthermore, recent light microscopic immunofluorescence studies reported that the β-subunit of human chorionic gonadotropin was located in the syncytiotrophoblast but human chorionic gonadotropin and its α-subunit were in both the layers.[4, 5]

For the demonstration of human chorionic gonadotropin at electron microscopic levels, the peroxidase-labeled antibody technique has been used previously.[6-9] However, these reports have been controversial as to the precise localization of this hormone in the trophoblastic cells. We demonstrate in this paper the ultrastructural localization of human chorionic gonadotropin in human placental chorionic villi by the application of the protein A–gold technique.[10]

From the Department of Obstetrics and Gynecology and the Department of Anatomy, University of Occupational and Environmental Health, School of Medicine.

Received for publication February 24, 1986; revised August 22, 1986; accepted August 27, 1986.

Reprint requests: Dr. Kunshige Hamasaki, Department of Obstetrics and Gynecology, University of Occupational and Environmental Health, School of Medicine, Yahatanishi-Ku, Kitakyushu 807, Japan.

Material and methods

Human placentas of 7, 9, and 12 weeks of gestation obtained from natural and elective abortions were used for the present study. For light microscopic observations, specimens were fixed in Bouin's solution for 24 hours at room temperature and routinely embedded in paraffin. Specimens for electron microscopy were fixed in Zamboni solution[11] for 18 hours at 4° C. After washing in 0.1 mol/L phosphate buffer containing 10% sucrose, most of them were postfixed with 1% osmium tetroxide in 0.1 mol/L phosphate buffer for 1 hour at 4° C. All specimens were dehydrated in graded concentrations of acetone and embedded in epoxy resin.

The unlabeled antibody peroxidase-antiperoxidase technique was used for the light microscopic immunocytochemistry. The working dilution of the antiserum against human chorionic gonadotropin (Dako Immunoglobulins) ranged from 1:500 to 1:2000. For the immunoelectron microscopy, the protein A–gold technique was used with some slight modifications.[12, 13] The etching treatment with saturated sodium metaperiodate was done for 1 hour only in osmium-postfixed specimens to restore the hormonal antigenicity. Ultrathin sections mounted on uncoated 150-mesh nickel grids were placed on 1% egg albumin in phosphate-buffered saline for 15 minutes and then incubated with specific rabbit antisera against human chorionic gonadotropin (Dako Immunoglobulins; UCB Bioproducts) for 2 hours. These two antisera did not show any

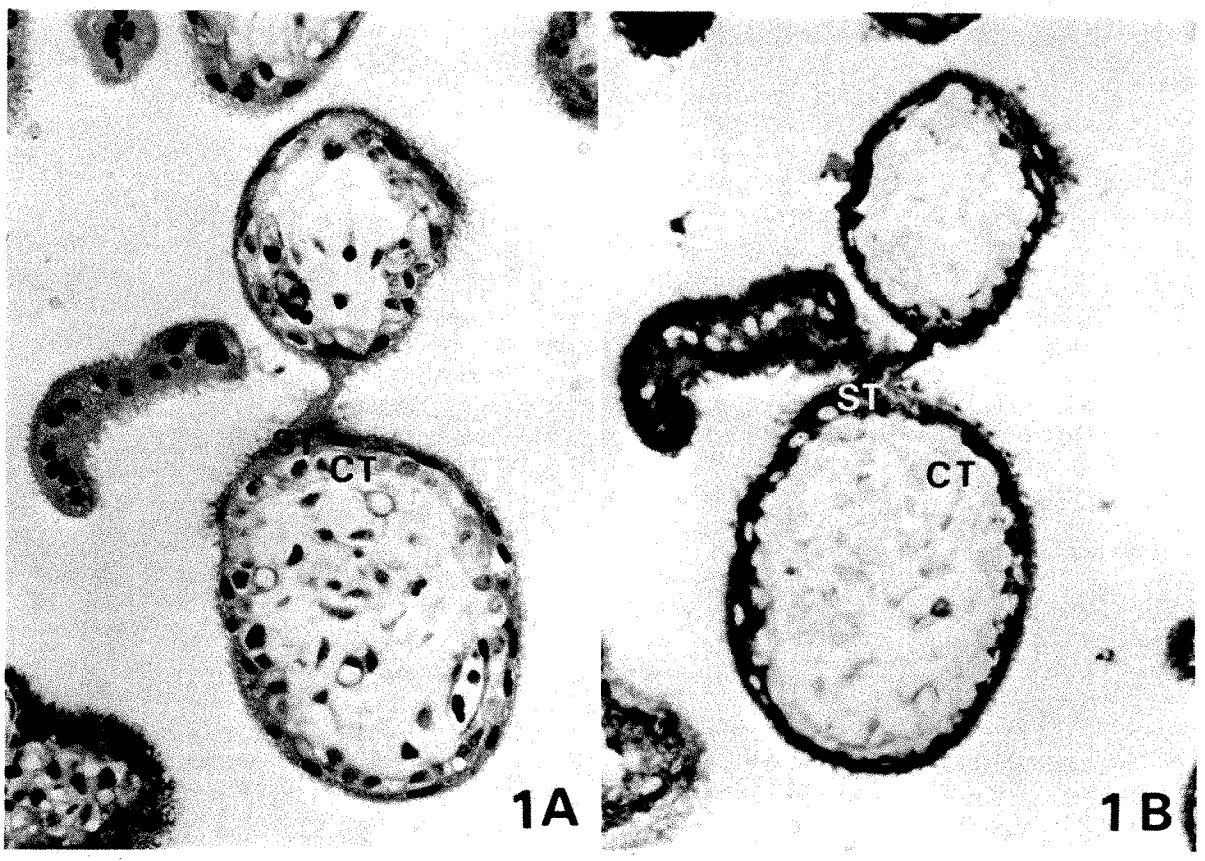


Fig. 1. Serial 4 μm sections of the chorionic villi at 7 weeks of gestation stained with Masson's trichrome *(A)* and with the peroxidase-antiperoxidase technique and anti–human chorionic gonadotropin serum *(B)*. Immunoreaction of human chorionic gonadotropin is localized only in the syncytiotrophoblast *(ST)*. *CT*, Cytotrophoblast. (×1300.)

difference in labeling intensity and specificity. The working dilution of the antisera ranged from 1:500 to 1:2000. After washing in phosphate-buffered saline for 15 minutes, the grids were placed on the protein A–gold solution diluted 1:10 in phosphate-buffered saline for 1 hour, stained with both uranyl acetate and lead nitrate, and examined with a JEM 100 CX electron microscope. The specificity of the immunolabeling was confirmed by the following controls: (1) replacement of the anti–human chorionic gonadotropin for normal rabbit serum or antisera against human prolactin, human growth hormone, and human adrenocorticotropic hormone (Dako Immunoglobulins) and (2) absorption of the anti–human chorionic gonadotropin with human chorionic gonadotropin (UCB Bioproducts).

Results

Fig. 1, *A*, shows human placental chorionic villi at 7 weeks of gestation stained with Masson's trichrome. In the adjacent 4 μm section incubated with anti–human chorionic gonadotropin at a concentration of 1:1000 and stained with the peroxidase-antiperoxidase technique, positive reactions of human chorionic gonadotropin were found exclusively in the syncytiotrophoblast (Fig. 1, *B*). Few nonspecific reactions were observed if normal rabbit serum or the other antisera (anti–human prolactin and anti–human growth hormone) were replaced for the specific antiserum.

In electron microscopy of the Zamboni-osmium–fixed sections at 7 weeks of gestation, the syncytiotrophoblast develops the Golgi apparatus and contains abundant membrane-bound inclusions of varying size and electron density, granules of 200 to 300 nm in diameter with moderate electron density *(MG)*, large bodies of 500 to 1000 nm *(LB)*, and large vacuoles of 500 to 1000 nm with various contents *(LV)* as shown in Fig. 2, *A* and *B*. Our electron micrographs show that the granules *(MG)* are derived from the Golgi apparatus *(GA*, Fig. 2, *A*) and aggregate to the apical cell surface (Fig. 2, *B*).

In the specimens labeled with the protein A–gold technique, the gold particles of 10 to 20 nm in diameter are preferentially concentrated on the Golgi-derived granules and large bodies (Fig. 3, *A* and *B*). The spec-

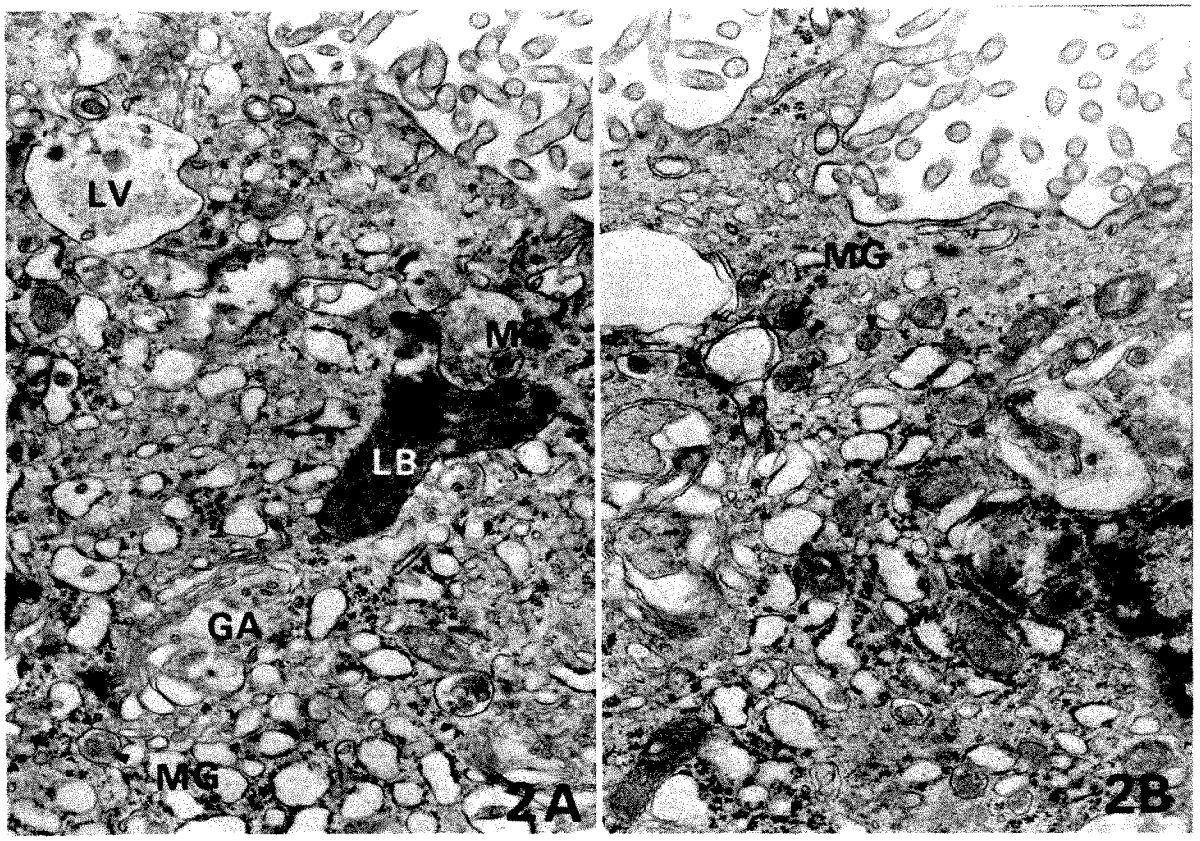


Fig. 2. Electron micrograph of the syncytiotrophoblast at 7 weeks of gestation showing three kinds of membrane-bound inclusions: granules of 200 to 300 nm with moderate electron density *(MG)*, large electron-dense bodies *(LB)*, and vacuoles *(LV)*. The granules *(arrowheads)* often exist near the Golgi apparatus *(GA)*, as shown in *A*, and are concentrated in the apical cell region, as shown in *B*. (Zamboni-osmium. *A*, ×23,000. *B*, ×25,000.)

imens fixed only in Zamboni solution remain more antigenic sites resulting in heavier labeling than those postfixed in osmium but yield much less satisfactory results, especially in the preservation of these inclusions (Fig. 4). Few nonspecific reactions were observed in the large vacuoles.

In the specimens at 9 and 12 weeks of gestation, these immunoreactive granular inclusions decrease considerably in number. Very few gold particles were observed on the cytoplasmic matrix and chorionic space in the controls when the specific anti–human chorionic gonadotropin serum was substituted for normal rabbit serum (Fig. 5, *A*) and the antiserum was absorbed with human chorionic gonadotropin (Fig. 5, *B*).

Comment

To date, four articles have described ultrastructural localizations of human chorionic gonadotropin in human placentas with the use of the peroxidase-antiperoxidase technique.[6-9] Although they reported localization of the reaction products either on the membranes of rough endoplasmic reticulum or in the cavities of apical vacuoles, the fine structure of these cytoplasmic organelles and inclusions was poorly preserved under methacrylate-embedded cryosections.

The present protein A–gold technique demonstrates that the antigenicity of human chorionic gonadotropin is preferentially located in Golgi-derived granules of 200 to 300 nm in the syncytiotrophoblast. From our electron micrographs, these granules seem to be segregated from the Golgi apparatus and released by exocytosis as secretory granules from the apical cell surface into the maternal circulation. Thus the present findings argue for the previous light microscopic immunocytochemical reports indicating human chorionic gonadotropin localization only in the syncytiotrophoblast.[2]

A few immunofluorescence studies reported that the β-subunit of human chorionic gonadotropin was also located in the cytotrophoblast.[4, 5] Therefore, we examined immunocytochemical localizations of α- and β-subunits of human chorionic gonadotropin by means of light microscopic peroxidase-antiperoxidase and immunoelectron microscopic protein A–gold techniques with the use of the specific antisera against both α- and

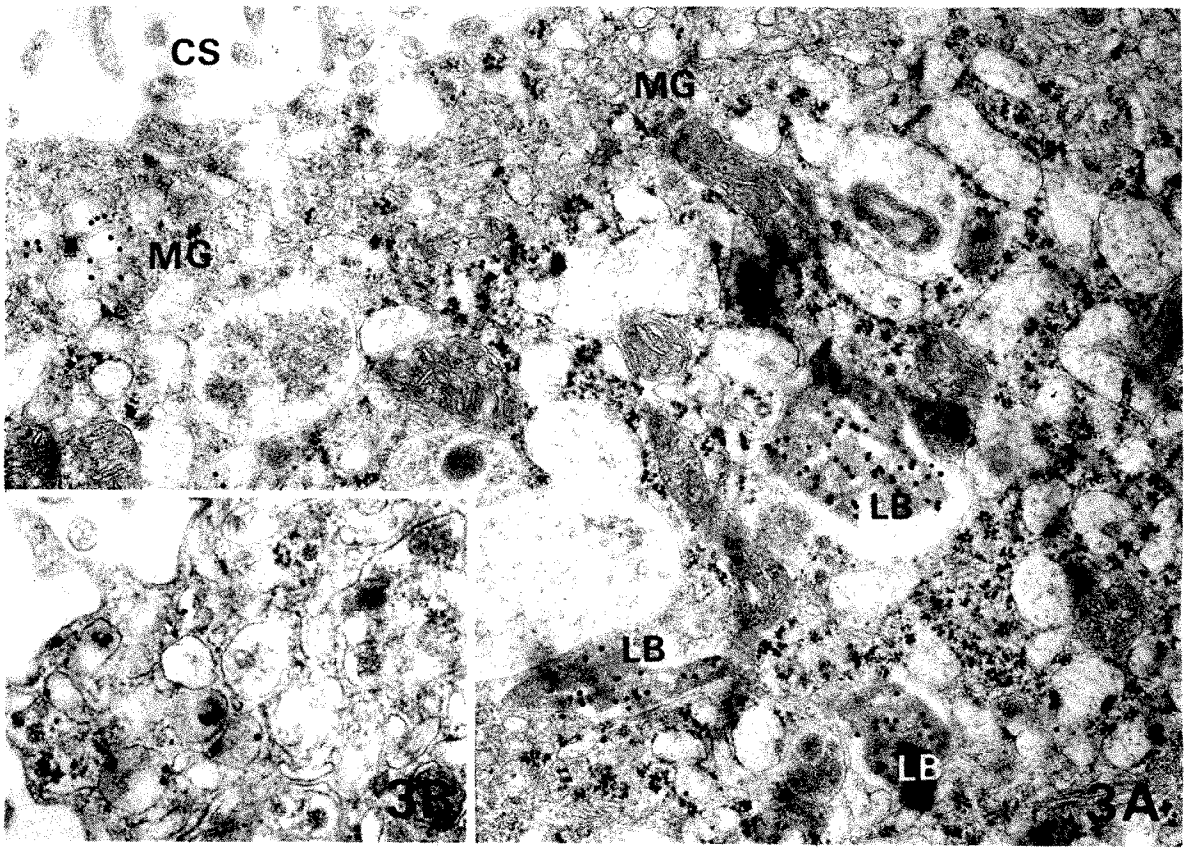


Fig. 3. Immunoelectron microscopic localization of human chorionic gonadotropin in the syncytiotrophoblast at 7 weeks of gestation. The gold particles are preferentially located on granules *(MG)* aggregating near the chorionic space *(CS)* and large bodies *(LB)*, as shown in *A*. The granules, although considerably reduced in electron density in this immunostaining section, are clearly distinguishable from the neighboring enlarged cisterns *(B)*. (Zamboni-osmium. *A*, ×29,000. *B*, ×40,000.)

β-subunits of human chorionic gonadotropin (UCB Bioproducts).[14] In both techniques, we did not obtain any specific labeling against these two antisera in the cytotrophoblast. Thus we conclude that the cytotrophoblast does not contain any immunospecificity against human chorionic gonadotropin and its subunits.

The present study reveals that the large body is another antigenic site of human chorionic gonadotropin in the syncytiotrophoblast. These do not seem to be secretory granules judging from their ultrastructure. Similarly, in the pituitary gland, it is well known that cells producing luteinizing hormone and follicle-stimulating hormone sometimes contain two kinds of granules, small secretory granules and large granules. The concentration of human chorionic gonadotropin in blood serum is high in the first trimester and rapidly declines thereafter.[15] Also, in the present observation, the granules have considerably decreased in number at 12 weeks. On these grounds, we speculate at present that the large bodies might play a role in reservoirs of the hormones during early gestation or in crinophagic lysosomal structures that digest the hormone as already mentioned in the prolactin cells.[16] At any rate, more detailed morphologic studies are necessary to elucidate the precise function of this inclusion in the syncytiotrophoblast, and such studies are now in progress in our laboratory.

REFERENCES

1. Pearse AGE. Cytochemistry of gonadotropic hormones. Nature 1943;162:651.
2. De Ikonicoff LK, Cedard L. Localization of human chorionic gonadotropic and sommatomammotropic hormones by the peroxidase immunohistoenzymologic method in villi and amnionic epithelium of human placentas (from six weeks to term). Am J Obstet Gynecol 1973;15:1124.
3. Fox H, Karkhongor FN. Immunofluorescent localization of chorionic gonadotropin in the placenta and in tissuse cultures of human trophoblast. J Pathol 1970;101:277.
4. Hoshina M, Ashitaka Y, Tojo S. Immunohistochemical interaction on antisera to HCG and its subunits with chorionic tissue of early gestation. Endocrinol Jpn 1979; 26:175.

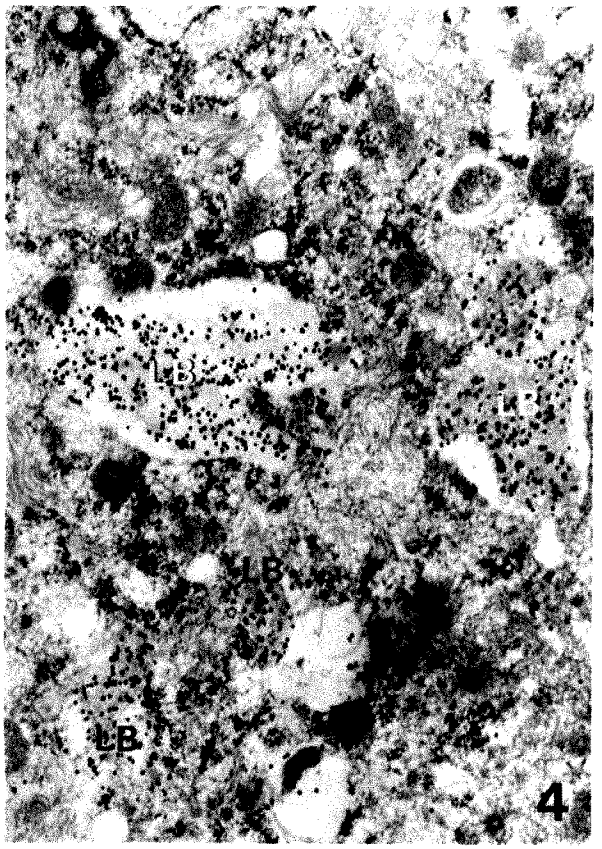


Fig. 4. Immunoelectron microscopic localization of human chorionic gonadotropin in the syncytiotrophoblast at 7 weeks of gestation fixed only in Zamboni solution. The heavy reaction of the gold particles is seen on large bodies *(LB)*. (×27,100.)

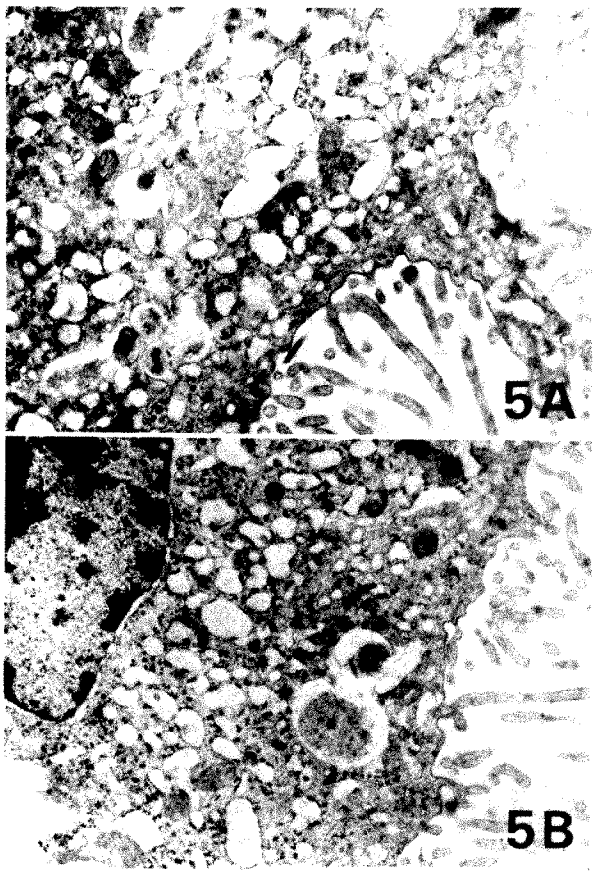


Fig. 5. Very few gold particles are present in the chorionic space and the cytoplasmic matrix of the syncytiotrophoblast at 7 weeks of gestation in the controls with substitution of the specific anti-human chorionic gonadotropin serum for normal rabbit serum *(A)* and absorption of the anti–human chorionic gonadotropin with human chorionic gonadotropin *(B)*. (Zamboni-osmium. ×13,500.)

5. Gaspard UJ, Hustin J, Reuter AM, Lambotte R, Franchimont P. Immunofluorescent localization of placental lactogen, chorionic gonadotrophin and its alpha and beta subunits in organ cultures of human placenta. Placenta 1980;1:135.
6. Dreskin RB, Spicer SS, Greene WG. Ultrastructural localization of chorionic gonadotropin in human term placenta. J Histochem Cytochem 1970;18:862.
7. Hamanaka N, Tanizawa O, Hashimoto T, Yoshinare S, Okudaira Y. Electron microscopic study on the localization of human chorionic gonadotropin (HCG) in the chorionic tissue by enzyme-labeled antibody technique. J Electron Microsc 1971;20:128.
8. Genbacev O, Robyn C, Pantic V. Localization of chorionic gonadotropin in human term placenta on ultrathin sections with peroxidase-labelled antibody. J Microsc 1972; 15:399.
9. Hayashi M. An immunohistochemical study of HCG and HPL on functional differentiation of human chorionic villi. Acta Obstet Gynecol Jpn 1980;32:94.
10. Roth J, Bendayan M, Orci L. Ultrastructural localization of intracellular antigens by the use of protein A-gold complex. J Histochem Cytochem 1978;20:1074.
11. Zamboni L, de Martino C. Buffered picric acid-formaldehyde: a new, rapid fixative for electron microscopy. J Cell Biol 1967;35:148A.
12. Ueda H, Fujimoto S. The protein A-gold technique: Use in the immunoelectron microscopic study of the teleost growth hormone. J Univ Occup Environ Health 1984; 6:39.
13. Ueda H, Kagawa H, Fujimoto S. Immunoelectron microscopic localization of growth hormone in the pituitary glands of two teleosts, tilapia *(Sarotherodon mossambicus)* and amago salmon *(Oncorhynchus rhodurus)*. Gen Comp Endocrinol 1985;59:149.
14. Fujimoto S, Ueda H, Hamasaki K. Immunocytochemical observations of human chorionic gonadotropin and human placental lactogen. In: Proceedings of the XII International Anatomical Congress 1985. London: Book Production Consultants, 1985;219.
15. Ashitaka Y, Nishimura R, Futamura K, Ohashi M, Tojo S. Serum and chorionic tissue concentrations of human chorionic gonadotropin and its subunits during pregnancy. Endocrinol Jpn 1974;21:547.
16. Farquhar M. Secretion and crinophagy in prolactin cells. In: Dellman HD, et al., eds. Comparative endocrinology of prolactin. New York: Plenum Press, 1977;37-94.

Nonsurgical therapy to preserve oviduct function in patients with tubal pregnancies

Kihyoe Ichinoe, M.D., Norio Wake, M.D., Naoko Shinkai, M.D., Yoshihiro Shiina, M.D., Yukio Miyazaki, M.D., and Toshinobu Tanaka, M.D.

Sapporo, Japan

Surgical treatment for tubal pregnancies greatly impairs the subsequent fertility of patients because of salpingectomy and its complicated adhesions. Nonsurgical methotrexate therapy was developed to avoid such complications. Although early detection was of prime importance, resolution of ectopic pregnancy was obtained in 22 patients (95.7%) with methotrexate administration alone. Patency of the oviducts was evaluated with hysterosalpingography and/or laparoscopy in 19 patients after termination of methotrexate treatment. In 10 of 19 patients (52.6%), complete patency of the involved oviduct confirmed the validity of this regimen. Severe side effects were not observed in any of the 23 patients. (Am J Obstet Gynecol 1987;156:484-7.)

Key words: Tubal pregnancy, methotrexate, salpingectomy

The term ectopic pregnancy is applied to a pregnancy that follows implantation of the fertilized egg on any tissue other than the mucous membrane lining of the uterine cavity. The most common type of ectopic pregnancy is that which occurs in the tube. Surgical treatment has been used once the tubal pregnancy is diagnosed. However, irrespective of the operative procedures, utilized, subsequent fertility has been impaired because of damage to the uninvolved tube. This damage has been assumed to be a sequel of the irritating factors of a blood clot in the peritoneal cavity and of operative procedures that cause fibrosis and obstructive adhesions of the oviduct. Thus excision of ectopic pregnancies or salpingostomy with reparative procedures has been used in an attempt to improve fertility. However, repeated tubal pregnancy in patients who underwent plastic surgery of the tube is not unusual.

The current availability of sophisticated clinical modes of diagnosis of pregnancy has also provided the possibility of early detection of ectopic pregnancy. Examinations by the use of B-scope scanning are of prime importance. The former is routinely applied for estimation of the date of conception and localization of the implantation site in all patients with early stage pregnancy. This system has made possible the early diagnosis of ectopic pregnancy before any signs of termination appear. Urinary and serum human chorionic gonadotropin measurements and endometrial curettage are also useful to establish the diagnosis in such cases.

Methotrexate is a folic acid antagonist that has been widely used for cancer treatment. Impairment of nucleic acid synthesis is responsible for the inhibition of tumor cell division. A similar effect was obtained with trophoblasts of abdominal pregnancies[1] since trophoblasts possess high proliferative activity. This idea has led to nonsurgical methotrexate treatment in patients with tubal pregnancy in order to conserve tubal function. In this study the efficacy of this treatment is discussed and the data obtained from 23 patients are analyzed.

Material and methods

Tubal pregnancy was diagnosed in 23 patients by means of B-scope scanning, urinary human chorionic gonadotropin measurements, endometrial currettage, and clinical course. The diagnosis was established in all cases before indications of termination of pregnancy. Methotrexate (0.4 mg/kg/day for 5 days) was administered intramuscularly to these patients. These courses were generally given every other week, although the schedule was occasionally altered by the degree of severity of side effects in the patients. The total dosage of methotrexate was determined with reference to the total urinary human chorionic gonadotropin titer in the patients measured by radioimmunoassay (Mochida Pharma. Co.). Resolution of tubal pregnancy was diagnosed in patients with urinary human chorionic gonadotropin levels that decreased to 20 IU/L. Patients were followed up for 2 to 3 months after the termination of methotrexate therapy. The patency of

From the Department of Obstetrics and Gynecology, Hokkaido University School of Medicine.

Received for publication November 12, 1985; revised August 5, 1986; accepted August 19, 1986.

Reprint requests: K. Ichinoe, M.D., Department of Obstetrics and Gynecology, Hokkaido University School of Medicine, Kita-ku, N-14, W-6 Sapporo 060, Japan.

Table I. Clinical data obtained from 22 patients with tubal pregnancy treated with methotrexate

Case No.	*Age (yr)*	*Site of previous ectopic gestation*	*Gestation (wk)*	*Human chorionic gonadotropin (IU/L)*	*Site of current ectopic gestation*	*Total dosage of methotrexate used (mg)*	*Length of time to resolution (days)*	*Complications*
1	25	—	9	80	?	75	6	—
2	41	Right tube	8	600	Left tube	105	23	—
3	20	—	7	1,000	Left tube	60	30	—
4	34	Left tube	9	1,000	Right tube	105	20	Impaired liver function
5	24	—	9	1,000	Right tube	100	14	—
6	25	—	12	2,000	Right tube	150	26	—
7	29	—	7	2,000	Left tube	180	28	—
8	27	Left tube	6	2,000	Right tube	200	29	—
9	29	—	7	4,000	?	200	38	Impaired liver function
10	37	—	8	4,000	Right tube	300	42	Impaired liver function
11	25	—	7	4,000	Right tube	200	43	Bone marrow suppression
12	25	Right tube	8	8,000	Left tube	200	21	Impaired liver function
13	29	Left tube	8	8,000	Right tube	75	39	—
14	21	—	8	16,000	Left tube	200	47	—
15	25	—	9	4,000	Right tube	200	—	—
16	34	Right tube	7	16,000	Left tube	200	44	—
17	23	—	5	320	Right tube	90	—	—
18	27	Left tube	8	640	Right tube	100	10	—
19	34	—	9	1,000	Left tube	105	17	—
20	25	Right tube	8	8,000	Left tube	200	25	—
21	24	—	9	640	Left tube	100	13	—
22	25	—	6	2,000	Left tube	180	7	—

the oviduct was evaluated by hysterosalpingography and/or laparoscopy.

Results

The ages of the 23 patients ranged from 20 to 41. Urinary human chorionic gonadotropin values in these patients were generally lower (80 to 16,000 IU/L) than those in intrauterine pregnancies (Table I). The left oviduct was involved in 11 cases and the right in 10 cases. The exact localization of the implantation site could not be confirmed in the remaining two cases. Methotrexate therapy was chosen for eight patients with a previous salpingectomy and in another 15 patients without such operation. Resolution was obtained in 22 patients with methotrexate administration alone. The remaining patient underwent immediate operation because of tubal rupture during the treatment. The total doses of methotrexate used in these 22 patients ranged from 60 to 300 mg (mean 151.1 mg). These doses tended to correspond to the urinary human chorionic gonadotropin titer before treatment; 60 to 150 mg (mean 93 mg) of methotrexate was sufficient to produce resolution in cases with urinary human chorionic gonadotropin levels <1000 IU/L, although 75 to 300 mg (mean 191 mg) was necessary in cases where the urinary human chorionic gonadotropin level was >2000 IU/L. The length of time required for the urinary human chorionic gonadotropin levels of patients to reach 20 IU/L with methotrexate treatment ranged from 6 to 47 days (mean 29.7 days). The mean in five patients with human chorionic gonadotropin titers <1000 IU/L before treatment was 18.6 days (6 to 30 days), although 35.9 days was necessary in 17 patients with levels >2000 IU/L.

The patency of bilateral oviducts was evaluated in 19 patients with hysterosalpingography and/or laparoscopy after termination of the methotrexate therapy (Table II). Those patients were then subdivided into two groups for exact evaluation, seven patients with a history of previous salpingectomy on either oviduct and 12 patients without such a history. Two patients (28.6%) had complete patency of the involved oviduct, although passage of the contrast medium was partially interrupted in three and completely interrupted in two patients in the former group. The patency of the uninvolved oviduct in the group without a history of salpingectomy could be demonstrated in all patients except in one case (Case 22) with peritubal adhesions. Normal passage of the contrast medium through the involved tube was observed in eight patients (58.3%), although obstruction and peritubal adhesions, respectively, were shown in two patients in this group. A high rate of impairment of passage through the involved oviduct was shown in the group with previous salpingectomy.

A further comparison of the rate of patency of the involved oviduct was performed between the group with early gestation (<7 weeks of pregnancy) and the group at a relatively advanced stage (>8 weeks of pregnancy). A higher success rate was obtained in the for-

Table II. Results of evaluation of tubal patency and pregnancies subsequent to methotrexate therapy in 22 patients with tubal pregnancy

Case No.	Tubal patency		Pregnancies subsequent to methotrexate therapy
	Involved tube	*Uninvolved tube*	
1	Good	Good	—
2	—	Not examined	—
3	Good	Good	Two normal neonates
4	Poor	—*	Induced abortion
5	—	Not examined	—
6	Good	Good	—
7	Good	Good	—
8	Poor	—*	—
9	Good	Good	—
10	—	Not examined	—
11	Obstruction	Good	—
12	Poor	—*	—
13	Obstruction	—*	—
14	Good	Good	—
15	Peritubal adhesions	Good	—
16	Good (obstruction)	—	—
17	Good	Peritubal adhesions	Spontaneous abortion at 5 wk, 3 days
18	Good	—*	—
19	Peritubal adhesions	Good	—
20	Good	—*	—
21	Obstruction	Good	—
22	Good	Peritubal adhesions	Normal neonate

*Cases in which salpingectomy was done for the previous tubal pregnancy.

mer (five of eight, 62.5%) than in the latter (five of 11, 45.5%), although the number of case evaluated was still small. The group without previous salpingectomy and with gestation of <7 weeks obtained the highest rate of patency of the involved oviduct (five of six, 83.3%). As a result, in 10 of 19 patients (52.6%) complete patency of the involved oviduct function was obtained with methotrexate administration alone.

Four patients had other pregnancies subsequent to the termination of methotrexate therapy in spite of the relatively short period of observation. Two patients (Cases 3 and 22) were delivered of three normal neonates although one (case 17) had a spontaneous abortion. The remaining patient (Case 4), who underwent elective abortion because of an economic problem, was especially noteworthy. Because the left tube of this patient had already been removed, a methotrexate regimen for treatment of a right tubal pregnancy was undertaken in the present study. This resulted in resolution of the tubal pregnancy, but there was impairment of passage of the contrast medium. However, she still retained reproductive capability.

Severe side effects were not observed in any of the 23 patients with administration of a total of 60 to 300 mg of methotrexate. Slight elevations of serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase, and slight bone marrow suppression were noted in four cases and one case, respectively.

Comment

Methotrexate treatment for extrauterine pregnancies was initially used in cases of abdominal pregnancy.[1-3] The prompt deterioration of the placenta produced by methotrexate administration may bring about resolution without sequelae, although surgical manipulation of the placenta has generally resulted in profuse hemorrhage. Similar trials undertaken for cervical and interstitial pregnancies also indicated the effectiveness of this therapy.[4] The rationale for the use of methotrexate in these instances was to bring about a prompt destruction of placental trophoblastic activity, with the assumption that this would be associated with a concomitant decrease in placental vascularity and thereby a marked restriction of the potential of hemorrhagic complications of the retained placenta. Methotrexate therapy for tubal pregnancies has also been based on the expectation of similar effects.

Surgical treatments greatly impair subsequent fertility in patients because of salpingectomy and its complicated adhesions. Simultaneous tuboplasty also frequently results in repeated tubal pregnancy. Nonsurgical methotrexate treatment was considered to avoid such complications. Although early detection is of prime importance in this respect, recent advances in B-scope scanning can produce the diagnosis in the stage before the pregnancy terminates. In fact, B-scope scanning resulted in early detection in 23 patients of the present study, and resolution occurred with methotrexate administration alone in all cases except one. However, preservation of the oviduct function should be also considered. In the present study, in 52.6% of the involved oviducts (10 tubes), complete patency occurred together with resolution of tubal pregnancy, thus confirming the clinical validity of this regimen.

The remaining nine tubes (47.4%) showed various degrees of interruption of passage on hysterosalpingography. The causal mechanisms responsible for this complication must be considered. In five patients with previous salpingectomy it is likely that the presence of peritubal adhesions, probably as the result of salpingectomy, caused the current tubal pregnancy as well as the passage interruption after the termination of methotrexate therapy. However, the details responsible for the complication remain to be clarified in the other four cases in which salpingectomy had not been performed. The erosive action of trophoblasts and the separation of the ovum from the tubal wall are sometimes re-

sponsible for bleeding into oviducts and from the fimbriated orifice, which may be filled with clots. Although the causal factors resulting in subsequent tubal pregnancy should be accounted for, it is likely that such bleeding into the oviduct gives rise to either peritubal adhesion or obstruction. This would suggest that methotrexate administration should be begun at the stage before the appearance of bleeding into the oviduct. The highest success rate obtained in the group without previous salpingectomy and in which the gestation was <7 weeks may support this idea. However, further accumulation of data is necessary to confirm the validity of this regimen.

The total dosage of methotrexate used in the present study was evidently less than the one used for patients with trophoblastic diseases. Only slight suppressions of liver and bone marrow functions have been observed. Those indicate the safety of methotrexate treatment for patients with tubal pregnancies, although imminent influences on offspring of women receiving methotrexate remain unknown. Literature on the topic[5-8] indicates that 3% to 10% of neonates born to mothers who had received chemotherapeutic regimens had congenital anomalies. These indications are at variance with our data (Shinkai N, Wake N, Ichinoe K, Shiina Y. unpublished data) that none of 53 patients had such anomalies. The cause of this difference is uncertain, but it is unlikely that a relationship exists between the incidence of congenital anomalies and the administration of chemotherapeutic agents because the latter has not been carried out in patients during pregnancy. Rather the degree of germ cell damage created by the agents is important to evaluate the safety of this regimen. This would be reflected by the rates of spontaneous abortion or some kinds of abnormal pregnancies. We are aware of 449 pregnancies after the termination of chemotherapy in the literature.[5-10] Sixty-five spontaneous abortions occurred, for a rate of 14.5%, which is not different from that in data of the control population without any kind of chemotherapy.[6-8] Thus previous data indicate that the rate of fetal wastage presumably resulting from the injured germ cells was not high, suggesting safety for offspring of women receiving methotrexate therapy. However, this should be investigated further since the available data are insufficient at this time.

REFERENCES

1. Hreshchyshyn MM, Naples JD Jr, Randall CL. Amethopterin in abdominal pregnancy. Am J Obstet Gynecol 1965;93:286-7.
2. Lathrop JC, Bowles GE. Methotrexate in abdominal pregnancy. Report of a case. Obstet Gynecol 1968;32:81-5.
3. St Clair JT, Whealer DA. Methotrexate in abdominal pregnancy. JAMA 1969;21:529-31.
4. Tanaka T, Hayashi H, Kutsuzawa T, Fujimoto S, Ichinoe K. Treatment of interstitial ectopic pregnancy with methotrexate: report of a successful case. Fertil Steril 1982;37:851-2.
5. Van Thiel DH, Ross GT, Lipsell MB. Pregnancies after chemotherapy of trophoblastic neoplasms. Science 1970; 16:1326-7.
6. Pastorfide GB, Goldstein DP. Pregnancy after hydatidiform mole. Obstet Gynecol 1973;42:67-70.
7. Ross GT. Congenital anomalies among children born of mothers receiving chemotherapy for gestational trophoblastic neoplasms. Cancer 1976;37:1043-7.
8. Walden PAM, Bagshawe KD. Reproductive performance of women successfully treated for gestational trophoblastic tumors. Am J Obstet Gynecol 1976;125:1108-14.
9. Kanazawa K, Moridaira H, Hirogami T, Hando T, Takeuchi S. Studies on treatment of invasive mole and subsequent reproductive performance, a report of 39 cases [In Japanese]. Acta Ostet Gynecol Jpn 1980;32:69-74.
10. Shinkai N, Shiina Y, Ichinoe K. Reproductive function in patients with trophoblastic disease [In Japanese]. Acta Obstet Gynecol Jpn 1981;33:301-4.

Bound volumes available to subscribers

Bound volumes of the American Journal of Obstetrics and Gynecology are available to subscribers (only) for the 1987 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 156 (January-June) and Vol. 157 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the Journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular Journal subscription.

Position Statement on Gestational Diabetes Mellitus

Formulated by the American Diabetes Association, Inc.

I. Definition, Detection, and Diagnosis
   A. Definition
      Gestational diabetes mellitus is defined as carbohydrate intolerance of variable severity with onset or first recognition during the present pregnancy. The definition applies regardless of whether insulin is used for treatment or the condition persists after pregnancy but does not exclude the possibility that the glucose intolerance may have antedated the pregnancy
   B. Detection
      All pregnant women should be screened for glucose intolerance because selective screening based on clinical attributes or past obstetric history has been shown to be inadequate. Pregnant women who have not been identified as having glucose intolerance before the twenty-fourth week should have a screening glucose load between the twenty-fourth and the twenty-eighth week consisting of 50 gm of oral glucose given without regard to time of the last meal or the time of day. The venous plasma glucose level is measured 1 hour later. A value of ≥140 mg/dl is recommended as a threshold to indicate the need for a full diagnostic glucose tolerance test
   C. Diagnosis
      Diagnosis is based on results of the 100 gm oral glucose tolerance test during pregnancy interpreted according to the diagnostic criteria of O'Sullivan and Mahan.[1] Definitive diagnosis requires that two or more of these venous plasma glucose concentrations be met or exceeded: fasting, 105 mg/dl; 1 hour, 190 mg/dl; 2 hours, 165 mg/dl; 3 hours, 145 mg/dl

II. Obstetric and Perinatal Considerations
   A. The offspring of mothers who experience fasting (≥105 mg/dl) and postprandial (≥120 mg/dl) glycemia are at greater risk for intrauterine or neonatal death, and such mothers must undergo careful antepartum fetal surveillance. Perinatal mortality in offspring of patients with gestational diabetes who maintain normal fasting and postprandial glucose levels may not be increased above that observed in the general population under conditions of *optimum* obstetric care and restitution of fasting and postprandial plasma glucose levels to normal limits. All patients with gestational diabetes are at significant risk for fetal macrosomia as well as other neonatal morbidity, including hypoglycemia, hypocalcemia, polycythemia, and hyperbilirubinemia

III. Therapeutic Strategies
   A. Close surveillance of mother and fetus must be maintained in all instances of gestational diabetes mellitus. Maternal surveillance should be directed toward monitoring for elevations of fasting or postprandial glucose levels in capillary blood or venous plasma and designed to detect any deterioration of glucose homeostasis as gestation proceeds
   B. Monitoring of maternal urinary glucose is no longer deemed adequate or sufficient
   C. Increased fetal surveillance is appropriate, but the starting time, frequency, and techniques used should depend on the cumulative degree of risk that the fetus is believed to bear
   D. All women with gestational diabetes mellitus should receive nutritional counseling consistent with the recommendations for caloric distribution proposed by the American Diabetes Association. Intake of sucrose should be limited
   E. If dietary management does not consistently maintain the fasting plasma glucose level ≤105 mg/dl and/or the 2-hour postprandial plasma glucose level ≤120 mg/dl on two or more occasions within a 2-week interval, insulin therapy is initiated in many centers. Self-monitoring of blood glucose is a requirement for effective insulin therapy. If insulin is prescribed, only highly purified nonbeef insulin preparations should be used
   F. Oral hypoglycemic agents cannot be recommended during pregnancy
   G. Breast-feeding should be encouraged in women with gestational diabetes mellitus

This statement is based on the summary and recommendations of the Second International Workshop-Conference on Gestational Diabetes Mellitus (published in Diabetes 1985;34:123-6). Approved by the Board of Directors of the American Diabetes Association, Inc., March 8, 1986, and reproduced with permission from the American Diabetes Association, Inc.

IV. Prognostic considerations
    A. Women in whom gestational diabetes mellitus is diagnosed should be evaluated post partum to detect diabetes early in its course. They should be evaluated initially at the first postpartum visit by a 2-hour oral glucose tolerance test with a 75 gm glucose load

REFERENCE

1. O'Sullivan JB, Mahan CM. Criteria for the oral glucose tolerance test in pregnancy. Diabetes 1964;13:278-85.

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

Human Papillomaviral Infection and Lower Genital Tract Neoplasia: An Advanced Course for Pathologists and Clinicians, May 7, 8, 9, 1987, Doubletree at Concourse Hotel, Atlanta, Georgia. Directed by Dr. Richard Reid and sponsored by The Regional Cancer Center–Saint Joseph's Hospital of Atlanta. For further information contact: Mary Claire Jones, Director of Educational Services. Tel.: (404) 851-7025.

High-Risk Obstetrics, April 2-4, 1987, Chicago, Illinois. Sponsored by the Cook County Graduate School of Medicine. For additional information contact the Registrar's Office. Toll-free number: Illinois 1 (800) 621-4649, outside Illinois 1 (800) 621-4651.

Current Concepts in Obstetrics and Gynecology Focusing on Endometriosis, March 27-28, 1987, Inn on the Park, Four Riverway, Houston, Texas. Presented by Baylor College of Medicine, Department of Obstetrics and Gynecology. For information contact: Lila K. Lerner, Program Coordinator, The Office of Continuing Education, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030. Tel.: (713) 799-6020.

The Annual International Reproductive Health Seminar, November 4-7, 1987, The Sheraton British Colonial, One Bay Street, Nassau, Bahamas. Sponsored by IPARC International Population and Reproduction Council. For further information contact: Howard A. Engle, M.D., Director General, IPARC, 975 Arthur Godfrey Road, Suite 102, Miami Beach, FL 33140. Tel.: (305) 531-0047.

Fourth Annual Houston Everett Memorial Course in OB-GYN Urology, Saturday, April 25, 1987, The Johns Hopkins Medical Institutions, Baltimore, Maryland. 9 AMA Category I credits; application has been made for other appropriate credits. Sponsored by the Department of Gynecology and Obstetrics, The Johns Hopkins Medical Institutions. For information contact: Program Coordinator, Office of Continuing Education, The Johns Hopkins Medical Institutions, Turner 22, 720 Rutland Ave., Baltimore, MD 21205. Tel.: (301) 955-6046.

The Pacific Coast Obstetrical and Gynecological Society Ted Adams Scholarship Committee invites applications for an award of $7,500 to be given in 1987 to a student, resident, or practicing physician residing in the geographical area of the Society (Alaska, Arizona, British Columbia, California, Hawaii, Idaho, Nevada, Oregon, Utah, and Washington). Applicants should submit a descriptive outline detailing their educational background, their present status, and how the award would be used in pursuit of knowledge that could elucidate and contribute to understanding of some aspect of normal or abnormal structural, functional, or behavioral component of human reproductive biology. All applications received by July 1, 1987, will be considered by the committee. The decision of the committee will be final and will be announced at the annual meeting of the Society, September 27 to October 2, 1987, at the Lodge at Pebble Beach, Pebble Beach, California. Address applications to: PCOGS Scholarship Committee, 707 Cobb Medical Center, Seattle, WA 98101.

Ninth Practical Course in Colposcopy and Cervicovaginal Histopathology in English. Demonstration of the use of Dr. Cartier's Diathermy Loop. Paris, France, September 21-25, 1987. For further information contact: Dr. R. Cartier, 20, rue des Cordelieres, F 75013 Paris, France. Tel.: (1) 43 36 04 46.

Reproductive Endocrinology and General Gynecology, May 31–June 6, 1987, Palmetto Dunes Resort, Hilton Head Island, South Carolina. Sponsored by the Department of Gynecology and Obstetrics, The Johns Hopkins Medical Institutions. Credits: 34 AMA. This program has been approved for 33 Cognates, Formal Learning, by the American College of Obstetricians and Gynecologists. For information contact: Jeanne Ryan, Program Coordinator, Office of Continuing Education, The Johns Hopkins Medical Institutions, 720 Rutland Ave., Turner 22, Baltimore, MD 21205. Tel.: (301) 955-6046.

The Role of β-Lactamase Inhibitor Combinations in the Management of Obstetric-Gynecologic Infections

Richard L. Sweet, M.D., *Chairman*
San Francisco, California

The following papers are selected from presentations given at the symposium, "The Role of β-Lactamase Inhibitor Combinations in the Management of Obstetric-Gynecologic Infections," held at the Beverly Wilshire Hotel, Beverly Hills, California, March 7-9, 1986.

This symposium was supported by a grant from Roerig, a division of Pfizer Pharmaceuticals.

Microbiology of the female genital tract

Ronald S. Gibbs, M.D.
San Antonio, Texas

Patients who contract genital tract infections are predominantly young, are otherwise healthy, and generally respond well to treatment for bacterial infections. These infections are most commonly polymicrobial in etiology, with several noteworthy exceptions. Often there is an inciting event such as childbirth, surgical intervention, pregnancy termination or intrauterine contraceptive device insertion. With treatment, prognosis for cure is excellent; however, sequelae such as recurrent infections, infertility, or ectopic pregnancy can be serious. Bacteria encountered in the female genital tract can be divided into aerobic and anaerobic organisms. Among the aerobic gram-positive organisms, several varieties of streptococci such as Group B streptococci and enterococci occur frequently. *Staphylococcus aureus* is an infrequent but important pathogen. Among the aerobic gram-negative organisms, the most common is *Escherichia coli. Klebsiella* sp. and *Proteus* sp. occur in about 5% of genital tract infections. Species that are more resistant to antibiotics, such as *Pseudomonas aeruginosa* and *Enterobacter* sp., occur in approximately 1% or 2% of these cases and are more likely to appear in patients who have previously received antibiotic therapy or who have been hospitalized for some time. Among the anaerobic organisms, the most common gram-positive isolates are *Peptostreptococci* and *Peptococci. Clostridia* sp. occurs less frequently. Among the anaerobic gram-negative organisms, the *Bacteroides* sp. most frequently encountered are *Bacteroides bivius* and *Bacteroides disiens. Bacteroides fragilis* is still a common problem but appears to be less predominent. Other organisms encountered are *Chlamydia trachomatis*, the genital mycoplasmas, yeasts, protozoa, and viruses. (Am J Obstet Gynecol 1987;156:491-5.)

Key words: Genital tract infections, female, microbiology, anaerobes and aerobes, sequelae

Surrounding the bacterial cell is a macromolecular matrix of peptidoglycan, or short strands of peptides cross-linked to a polysaccharide polymer. In gram-positive organisms, this matrix is covalently bound to teichoic acid or teichoglucuronic acid, while in gram-negative organisms, it is bound to the lipoprotein in the outer membrane. New cell walls are developed by the cross-linking of these adjacent peptides, thought to be the sites of β-lactam antibiotic action. This activity against either gram-negative or gram-positive species is dependent on the ability of the β-lactam antibiotic to penetrate through the peptidoglycan matrix and attach to a receptor site. Next, the β-lactam antibiotics must be resistant to β-lactamases and must have an affinity

From the Department of Obstetrics and Gynecology, University of Texas Health Science Center.
Reprint requests: Dr. Ronald S. Gibbs, University of Texas Health Science Center, 7703 Floyd Curl Dr., Room 439D, San Antonio, TX 78234.

for the penicillin-binding proteins involved in the synthesis of the bacterial cell wall.

Group B streptococci

The group B streptococci are representative of the aerobic gram-positive cocci. They are isolated in selective media from 5% to 25% of the vaginal cultures of otherwise healthy women. They are commonly involved in maternal and neonatal infections but play less of a role in gynecologic infections. They are involved in endometritis, amnionitis, and neonatal sepsis.

Group B streptococci are encountered in approximately 20% of obstetric infections, and in neonatal sepsis they are one of the most common organisms isolated from the bloodstream.[1] An association has been shown between colonization of the maternal cervix in pregnancy and subsequent adverse outcomes of pregnancy, such as premature rupture of the membranes or premature birth.[2]

The major current problem with group B streptococci is the prediction and prevention of early-onset neonatal sepsis. Even with prompt treatment, the mortality rate is approximately 40%. Boyer et al.[3] have reported using selective intrapartum chemotherapy in women who are colonized with group B streptococci during pregnancy and who have additional risk factors such as premature rupture of the membranes, premature labor, or maternal fever in labor. This treatment has previously been reported to decrease transmission to the neonate and very recently has been reported to significantly decrease group B streptococcal neonatal sepsis.[4]

Group B streptococci are widely susceptible to β-lactam antibiotics, including the penicillins and cephalosporins. Alternative therapy for treating group B streptococcal infection includes erythromycin.

Enterococci

Enterococci are also common gram-positive cocci, the most common being *Streptococcus faecalis*. These are included in the group D streptococci in the Landsfield system. They are isolated from the genital tract of approximately 10% of asymptomatic women. Enterococci are occasionally pathogenic in the heart valve and urinary tract.

During the last decade, the role of enterococci in pelvic infections has been controversial. Isolated from cultures of infected sites in women with endometritis, amnionitis, and postoperative gynecologic infections, they have also been isolated in the bloodstream of women with genital tract infections. Apparently the frequency of isolation of this organism is increasing and it is now found in approximately 25% of the women with postoperative infection after cesarean section.[5] Use of prophylactic cephalosporins appears to be a potent method of increasing the isolation rate of enterococci.[6]

The treatment of choice of *S. faecalis* is a synergistic combination of penicillin plus an aminoglycoside such as gentamicin. In a penicillin-allergic individual, vancomycin may be used as the drug of choice. Patients who have enterococcal urinary tract infections or mild enterococcal infections usually respond well to ampicillin therapy. However, the organism is resistant to penicillin alone, gentamicin alone, and all the cephalosporins including those of the first, second, and third generations, as well as the tetracyclines. When sulbactam is combined with cefoperazone, the 90% minimum inhibitory concentration with enterococci does not decrease markedly.[7]

Staphylococcus aureus

S. aureus is found in the genital tract of approximately 9% to 10% of asymptomatic women, in approximately 10% of patients with postoperative wound abscess after gynecologic or obstetric procedures, and in 5% to 20% of genital tract cultures of women with pelvic infection. It is found in virtually 100% of women who have toxic shock syndrome.[8]

Forty years ago widespread resistance to penicillin G developed in nosocomial isolates of *S. aureus*, and currently 95% of hospital isolates and approximately 85% of community isolates of these organisms produce β-lactamases that confer resistance to penicillin and ampicillin as well as the newer penicillins. Staphylococci are usually susceptible to the β-lactamase–resistant penicillins in the methicillin-nafcillin group, to cephalosporins, and to clindamycin. Methicillin-resistant strains of *S. aureus* have been a problem outside the United States and a relatively infrequent but perhaps growing problem here.

Escherichia coli

E. coli is the most common aerobic gram-negative bacterium in the female pelvis.[9] It is the most common organism isolated from the bloodstream of women who have septic shock complicating pelvic infections.[9] However, it is pathogenic in only 10% to 20% of pelvic infections.[9] This estimate is based on studies with agents such as metronidazole or clindamycin, neither of which are active against *E. coli* or other Enterobacteriaciae.

E. coli and other members of this group are often susceptible to β-lactam antibiotics including several of the newer penicillins and cephalosporins. Aminoglycosides are, however, the drugs of choice.

Several different β-lactamases are detectable in the Enterobacteriaceae group, including *E. coli*. TEM-1 is the most common and accounts for roughly 75% of the β-lactamases produced by this group.[10] These TEM enzymes are plasmid-mediated and constitutive.

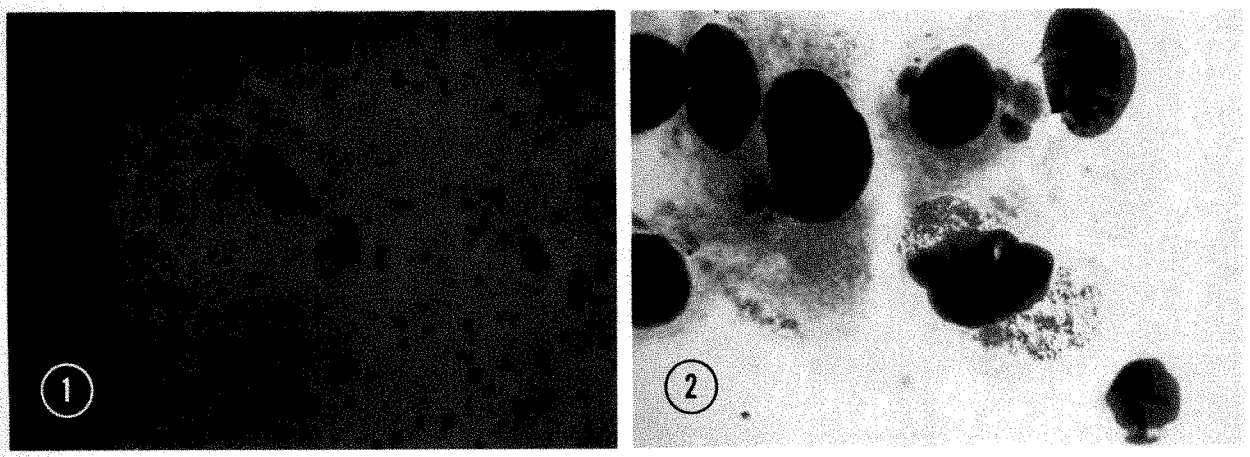


Fig. 1. A culture showing genital mycoplasmas. The larger "fried egg" colonies are *M. hominis*, and the smaller colonies are *U. urealyticum*.

Fig. 2. Tissue culture of *C. trachomatis*. Stain demonstrates *C. trachomatis* inclusions.

Neisseria gonorrhoeae

N. gonorrhoeae is an organism of preeminent importance in pelvic inflammatory disease and may cause disseminated infection, especially in pregnant women. It is isolated in 1% to 25% of cultures depending on the population screened.[11]

Traditionally, *N. gonorrhoeae* has been susceptible to penicillins and other β-lactam antibiotics, as well as to tetracyclines. A little more than a decade ago, however, penicillin-producing strains of *N. gonorrhoeae* were recognized, primarily in the Far East. These strains, however, have remained at a relatively low prevalence in the United States. The β-lactamases responsible for penicillin resistance in *N. gonorrhoeae* are also of the TEM 1 type.

Peptostreptococci

Peptostreptococci are among the most common anaerobic organisms encountered in female genital tract infections and have been isolated, according to recent reports, from 20% to 60% of genital tract cultures.

Peptostreptococci have generally remained widely susceptible to antibiotics, including penicillins, clindamycin, metronidazole, and cephalosporins. Few strains of peptostreptococci have minimum inhibitory concentrations ≥64 μg/ml of penicillin.

Bacteroides bivius

Much information has been gathered concerning the *Bacteroides* sp. isolated from female genital infections. First recognized in 1977, *Bacteroides bivius* is representative of these anaerobic gram-negative bacteria. Several recent studies have determined *B. bivius* to be the most common anaerobe in female genital infections. We find it in approximately 20% of women who have endometritis after cesarean section and in the amniotic fluid of about 20% of women with intra-amniotic infection.[12] In other series it has been isolated in up to 40% of genital tract cultures from women with pelvic infection.

B. bivius is highly susceptible to clindamycin, metronidazole, some of the newer penicillins, and the newer cephalosporins. However, susceptibility to penicillin G is highly variable. Ranges of minimum inhibitory concentrations are broad, and in three susceptibility studies of *B. bivius* the 90% minimum inhibitory concentration values have been 16 to 64 μg/ml.[13-15]

Bacteroides disiens is another recently recognized species found mainly in female genital tract infections. Found in from 2% to approximately 15% of cultures from infected genital tract sites, it has susceptibility patterns similar to those seen with *B. bivius*.[13-15]

Bacteroides fragilis is found in female genital tract infections, but this group is found less commonly than *B. bivius* or *B. disiens* according to recent reports. Approximately 90% of the strains in the *B. fragilis* group produce a constitutive β-lactamase. Agents of choice for treating *B. fragilis* include clindamycin, metronidazole, chloramphenicol, and cefoxitin.

Mycoplasmas

Fig. 1 shows cultures of genital mycoplasmas. *Mycoplasma hominis* is found in 30% to 50% of vaginal cultures of sexually active women, and serologic and blood culture evidence supports a role for this organism in both intrapartum and postpartum infection.[16-18] It is susceptible to tetracyclines and to clindamycin, while β-lactam antibiotics tend to be rather inactive.

The other common genital mycoplasma is *Ureaplasma urealyticum*, being more common in vaginal cultures than *M. hominis*. It is found in 60% to 80% of vaginal cultures of sexually active women. *U. urealyticum* has

been implicated for several decades as a cause of infertility, pregnancy wastage, and premature birth. However, evidence is stronger for the role of *U. urealyticum* in postpartum fever. We have not been able to discern a role for *Ureaplasma* in intra-amniotic infections. Drugs of choice for treating *U. urealyticum* remain tetracycline and erythromycin.

Chlamydia trachomatis

Fig. 2 shows a culture of *C. trachomatis*. It is found in from 2% to 25% of cervical cultures, the wide range being a function of the population. *Chlamydia* is a major cause of acute cervicitis and can cause salpingitis.

Its role in endometritis, however, is less clear. Recent studies from Chicago as well as those from Seattle have implied its role in late-onset, mild endometritis after vaginal delivery.[19, 20] The organism is also an important cause of perinatally acquired infections, neonatal conjunctivitis, and pneumonia.

Agents of choice for treating *Chlamydia* include the tetracyclines, erythromycin, and sulfonamides. Some β-lactam antibiotics, including amoxicillin and ampicillin, have moderate degrees of activity.

Viruses

The viruses that occur commonly in the female genital tract include herpes simplex virus, which has an incidence of less than 1%. Ninety percent of these isolates are of type 2. Cytomegalovirus is most common, being found in some 4% to 15% of the female genital tracts examined. Disseminated infection and viremias from this virus may play an important role in pregnancy infections.

Yeasts

Yeasts are commonly encountered in the genital tract and nearly all are of *Candida* sp. About 95% of all *Candida* sp. found are *Candida albicans*. Approximately 20% of asymptomatic, sexually active women are found to have yeast forms, responsible for about 25% of all cases of vaginitis.[21]

The response rate of these infections is usually good to a variety of compounds including boric acid, gentian violet, and imidazoles. However, the major problem with yeast infections is recurrence and reinfection. Disseminated candidal infections are unusual in otherwise healthy women.

Trichomonas vaginalis

The protozoan encountered commonly in the female genital tract infection is *T. vaginalis*. It is found in approximately 6% to 44% of women.[22] The higher end of the range is usually seen in women attending clinics for sexually transmitted diseases. This organism is responsible for approximately 20% of all infectious vaginitis. The response to treatment with single-dose metronidazole is usually good.[23]

Comment

Many infections of the female genital tract are polymicrobial. Both anaerobes and aerobes have been implicated in these infections. In a rat peritonitis model, organisms of the *E. coli* group, Enterobacteriaceae, contribute to peritonitis, to sepsis, and to death, but it is the anaerobic organisms that contribute to abscess formation.[24]

It has been shown in the rat model, as well as in more recent clinical trials of female patients with genital tract infections, that therapy should be aimed at both the aerobes and anaerobes.[25] The overall response rate will be better, and the likelihood of serious complications such as abscess or septic pelvic thrombophlebitis will be decreased. This was first demonstrated in a study of penicillin and gentamicin compared with clindamycin and gentamicin.[25] The response rate to clindamycin and gentamicin was significantly better and serious complications were decreased.

Single-agent therapy, as with cefoxitin, cefotaxime, moxalactam, and others that have very good activity in vitro against aerobes and anaerobes, has been reported to provide a similar high response rate.[5, 26-28]

REFERENCES

1. Baker CJ, Edwards MS. Group B streptococcal infections. In: Remington JS, Klein JO, eds. Infectious diseases of the fetus and newborn infant. Philadelphia: WB Saunders, 1983:820.
2. Regan JA, Chao S, James LS. Premature rupture of membranes, preterm delivery, and group B streptococcal colonization of mothers. Am J Obstet Gynecol 1980; 141:184.
3. Boyer KM, Gadzala CA, Kelly PD, et al. Selective intrapartum chemoprophylaxis of neonatal group B streptococcal early-onset disease. III. Interruption of mother-to-infant transmission. J Infect Dis 1983;148:810.
4. Boyer MM, Gotoff SP. Prevention of early-onset neonatal group B streptococcal disease with selective intrapartum chemoprophylaxis. N Engl J Med 1986;314:1665.
5. Gibbs RS, Blanco JD, Duff P, et al. A double-blind, randomized comparison of moxalactam versus clindamycin-gentamicin in treatment of endomyometritis after cesarean section delivery. Am J Obstet Gynecol 1983;146: 769.
6. Gibbs RS, St Clair PJ, Castillo MS, et al. Bacteriologic effects of antibiotic prophylaxis in high-risk cesarean section. Obstet Gynecol 1981;57:277.
7. Jones RN, Barry AL, Thornsberry C, et al. The cefoperazone-sulbactam combination: in vitro qualities including beta-lactamase stability, antimicrobial activity, and interpretive criteria for disk diffusion tests. Am J Clin Pathol 1985;84:496.
8. Wager GP. Toxic shock syndrome: a review. Am J Obstet Gynecol 1983;146:93.
9. Gibbs RS, Blanco JD, Bernstein S. Role of aerobic gram-negative bacilli in endometritis after cesarean section. Rev Infect Dis 1985;7:S690.
10. Neu HC. Contribution of beta-lactamases to bacterial resistance and mechanisms to inhibit beta-lactamases. Am J Med 1985;79(suppl 5B):2.

11. United States Department of Health and Human Services, Public Health Service. Basic statistics in STD problems in the U.S. In: STD fact sheet. ed 35. Bethesda, Maryland: National Library of Medicine, 1981; HHS publication no. (CCDC)81-8195.
12. Gibbs RS, Blanco JD, St Clair PJ, et al. Quantitative bacteriology of amniotic fluid from women with clinical intraamniotic infection at term. J Infect Dis 1982;145:1.
13. Ohm-Smith MJ, Hadley WK, Sweet RL. In vitro activity of new beta-lactam antibiotics and other antimicrobial drugs against anaerobic isolates from obstetric and gynecologic infections. Antimicrob Agents Chemother 1982;22:711.
14. Hill GB, Ayers OM. Antimicrobial susceptibilities of anaerobic bacteria isolated from female genital tract infections. Antimicrob Agents Chemother 1985;27:324.
15. Snydman DR, Tally FP, Knuppel R, et al. *Bacteroides bivius* and *Bacteroides disiens* in obstetrical patients: clinical findings and antimicrobial susceptibilities. J Antimicrob Chemother 1980;6:519.
16. Gibbs RS, Cassell GH, Davis JK, et al. Further studies on genital mycoplasms in intra-amniotic infection: blood cultures and serologic response. AM J OBSTET GYNECOL 1986;154:717.
17. Lamey JR, Eschenbach DA, Mitchell SH, et al. Isolation of mycoplasmas and bacteria from the blood of postpartum women. AM J OBSTET GYNECOL 1982;143:104.
18. Wallace RJ, Alpert S, Browne K, et al. Isolation of *Mycoplasma hominis* from blood cultures in patients with postpartum fever. Obstet Gynecol 1978;51:181.
19. Wager GP, Martin DH, Koutsky L, et al. Puerperal infectious morbidity: relationship to route of delivery and to antepartum *Chlamydia trachomatis* infection. AM J OBSTET GYNECOL 1980;138:1028.
20. Ismail MA, Chandler AE, Beem MO, et al. Chlamydial colonization of the cervix in pregnant adolescents. J Reprod Med 1985;30:549.
21. Gardner HL, Dukes CD. *Haemophilus vaginalis* vaginitis: a newly defined specific infection previously classified "nonspecific" vaginitis. AM J OBSTET GYNECOL 1955;69:962.
22. McLellan R, Spence MR, Brockman M, et al. The clinical diagnosis of trichomoniasis. Obstet Gynecol 1982;60:30.
23. Hager WD, Brown ST, Kraus SJ, et al. Metronidazole for vaginal trichomoniasis: Seven-day vs single-dose regimens. JAMA 1980;244:1219.
24. Weinstein WM, Onderdonk AB, Bartlett JG, et al. Experimental intra-abdominal abscesses in rats: development of an experimental model. Infect Immun 1974;10:1250.
25. diZerega G, Yonekura L, Roy S, et al. A comparison of clindamycin-gentamicin and penicillin-gentamicin in the treatment of post–cesarean section endomyometritis. AM J OBSTET GYNECOL 1979;134:238.
26. Sweet RL, Ledger WJ. Cefoxitin: single-agent treatment of mixed aerobic-anaerobic pelvic infections. Obstet Gynecol 1979;54:193.
27. Hemsell DL, Cunningham FG, DePalma RT, et al. Cefotaxime sodium therapy for endomyometritis following cesarean section: dose-finding and comparative studies. Obstet Gynecol 1983;62:489.
28. Sweet RL, Robbie MO, Ohm-Smith M, et al. Comparative study of piperacillin versus cefoxitin in the treatment of obstetric and gynecologic infections. AM J OBSTET GYNECOL 1983;145:342.

A review of the role of β-lactamase–producing bacteria in obstetric-gynecologic infections

David A. Eschenbach, M.D.

Seattle, Washington

β-Lactam antibiotics are the most commonly used antibiotics in obstetrics and gynecology. However, they are susceptible to inactivation when attacked by β-lactamase, an enzyme produced by many bacterial species. During the past three decades, numerous penicillins and cephalosporins have been made with a stable β-lactam ring that resists enzyme attack. More recently enzyme inhibitors have been discovered that inactivate β-lactamase. The combination of an enzyme inhibitor with a β-lactam antibiotic, such as ampicillin, restores the antimicrobial activity of the β-lactam against formerly resistant strains of staphylococci, *Haemophilus influenzae*, Enterobacteriaceae, and *Bacteroides fragilis*. (AM J OBSTET GYNECOL 1987;156:495-503.)

Key words: β-Lactam antibiotic, β-lactamase inhibitor, antibiotic resistance, sulbactam

Recognition of antibiotic resistance began shortly after the introduction of antibiotic therapy in the 1940s and it has become increasingly important today.[1] The rapid and widespread resistance of *Staphylococcus aureus* to penicillin is well documented. Currently more than 95% of *S. aureus* isolates produce β-lactamase enzymes that confer resistance to penicillin and ampicillin. Increasing ampicillin resistance has also been noted for the Enterobacteriaceae including *Escherichia coli*, *Kleb-*

From the Department of Obstetrics and Gynecology, University of Washington.
Reprint requests: David A. Eschenbach, M.D., Department of Obstetrics and Gynecology, RH-20, University of Washington, Seattle, WA 98195.

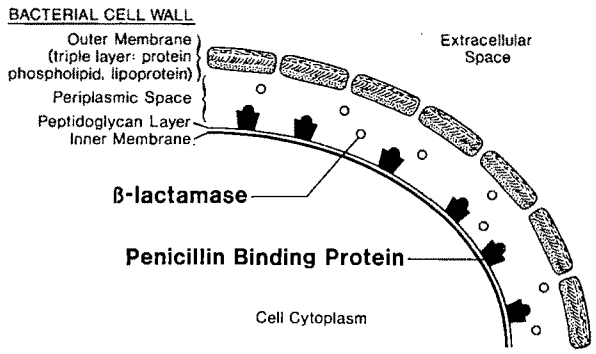


Fig. 1. Schematic representation of gram-negative bacterial cell envelope.

siella pneumoniae, and *Proteus mirabilis.*[2] *Haemophilus influenzae* resistance to ampicillin is now common. *Bacteroides fragilis* and a variety of other *Bacteroides* sp. have long been recognized to be resistant to many penicillins and cephalosporins. The ability of certain organisms to resist the action of β-lactam antibiotics by producing β-lactamase has led to well-documented therapeutic failures to eradicate the organism.[3]

Resistance has generally followed the widespread use of new agents. Some organisms are more able to acquire resistance than others. The most common mechanism of resistance involves bacterial elaboration of antibiotic-inactivating enzymes called β-lactamases. For example, *S. aureus* and Enterobacteriaceae commonly produce β-lactamase that makes them resistant to penicillin. The ability to elaborate β-lactamase enzymes is most commonly acquired when new genetic material is gained by the bacteria through transfer of plasmids from other β-lactamase–producing bacteria.[4] β-Lactamase can also be acquired through random chromosomal mutation of bacteria followed by the selection of resistant organisms through the use of antibiotics.

Other mechanisms of bacterial resistance are present for non–β-lactam antibiotics and, depending on the organism, these other mechanisms of resistance can also inhibit some β-lactam antibiotics. These mechanisms do not involve enzymatic inactivation. Nonenzymatic inactivation of an antibiotic can occur through alteration of the antibiotic target site on the bacterial cell, by blocking antibiotic transport within the bacteria or by bypassing a metabolic step inhibited by the antibiotic. The first two mechanisms change the ability of the drug to reach its site of activity within the bacteria. Examples of this resistance include *S. aureus* resistance to methicillin. A less well-known form of resistance is tolerance, in which organisms that are susceptible to antibiotic inhibition in vitro are still able to tolerate the bactericidal (killing) action of penicillin or cephalosporin.[5] These other mechanisms of resistance will be discussed briefly below to put bacterial resistance in perspective, but the report will emphasize β-lactamase resistance.

Mechanism of action of β-lactam antibiotics

β-Lactam antibiotics are the most widely used antibiotics. They make up more than one third of the top 10 drugs sold in the United States. From 20% to 35% of the typical hospital budget for drugs is spent on cephalosporins alone.[6] They enjoy frequent use both because of their effectiveness and because of their low toxicity. Penicillins, cephalosporins, and cephamycins exert their bactericidal activities by a similar mechanism. These β-lactam antibiotics inhibit bacterial cell wall synthesis, which ultimately produces cell lysis and death.

The basic penicillin nucleus contains a four-membered β-lactam ring attached to a five-membered thiazolidine ring. Penicillin and its derivatives differ in the structures of their side (R) chains. The structure of the basic cephalosporin nucleus contains the same four-membered β-lactam ring as penicillin. However, cephalosporins have a six-membered dihydrothiazide ring attached instead of the thiazolidine ring. Cephalosporins differ in the structures of the two (x and y) side chains.

It is well known that the cell wall is essential for the normal growth and integrity of bacteria. A cross-linked peptidoglycan heteropolymer confers the property of cell wall rigidity necessary for osmotic stability of the bacteria. Penicillin and other β-lactam antibiotics block cell wall synthesis by inhibiting transpeptidase cross-linking, causing weakness of the wall. A combination of mechanical and osmotic instability acts to cause cell death.[7]

However, a critical step that must occur before β-lactam antibiotics are active is the binding of the antibiotic to penicillin-binding proteins (Fig. 1). The antibiotic in the serum reaches the extracellular space between bacteria and it penetrates through pores in the outer membrane of the cell to reach the periplasmic space between the outer and inner cell wall. The inner membrane of the cell surrounds the cytoplasm. Penicillin-binding proteins project into the periplasmic space from their attachment to the inner cell membrane. The β-lactam antibiotic in the periplasmic space must attach to the penicillin-binding protein before inhibition of cell wall synthesis can occur. Inhibition of cell wall synthesis prevents growth of new bacteria and, as previously mentioned, it also causes cell lysis by causing mechanical and osmotic instability.

Cell lysis can also occur directly with higher concentrations of β-lactam antibiotics through activation of cellular autolysins. Although an endogenous intracel-

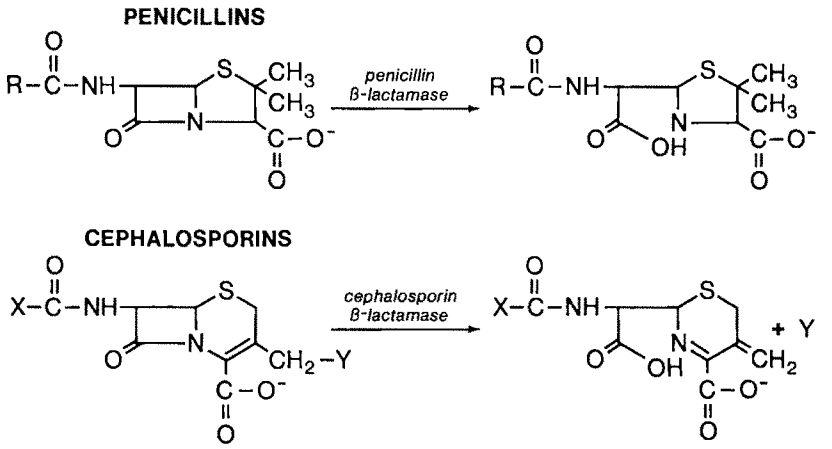


Fig. 2. Action of β-lactamase upon penicillins and cephalosporins, showing the hydrolysis or opening of the β-lactam ring.

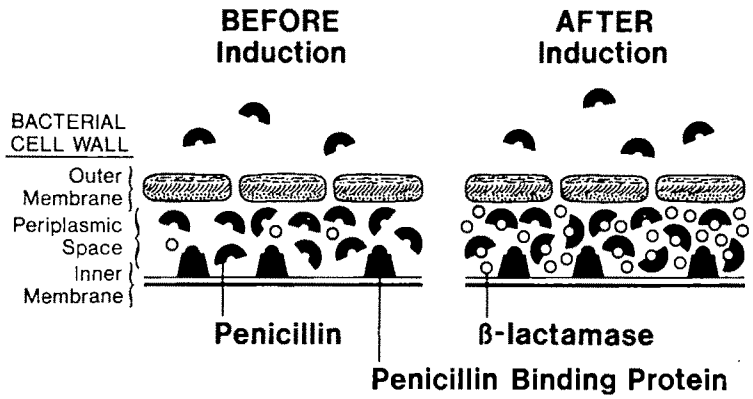


Fig. 3. The presence of β-lactamase in the periplasmic space before and after induction. Greater amounts of β-lactamase result in higher rates of hydrolysis and increased binding of β-lactamase to the β-lactam antibiotic. In both cases less drug is available to bind to penicillin-binding proteins necessary for antibiotic action.

lular inhibitor usually prevents cell destruction by autolysins, β-lactams can allow autolysins to come into contact with their substrates (peptidoglycans), which causes cell lysis and subsequent cell death.[7]

Mechanisms of organism resistance to β-lactamase

There are several mechanisms by which organisms are able to resist the action of β-lactam antibiotics that differ, depending on the antibiotic and the organism. As mentioned, the enzymatic destruction of an antibiotic is the most common and clinically significant manner of antibiotic resistance. Of the three possible enzymes that can potentially inactivate antibiotics—β-lactamase, amidase, and esterase—only β-lactamase is considered to be clinically important. β-Lactamases are present in a wide variety of both gram-positive and gram-negative bacteria. The enzyme irreversibly disrupts the amide bond within the β-lactam ring. Since the β-lactam ring is the crucial site that confers antibacterial activity, opening of the ring causes the antibiotic to lose activity (Fig. 2).

β-Lactamase is located in the periplasmic space between the penicillin-binding protein on the inner membrane and the outer cell membrane. Thus β-lactamases surround the penicillin-binding protein sites. When penicillin penetrates through the outer membrane to reach the periplasmic space, it is susceptible to the action of β-lactamase in the periplasmic space. If β-lactamase is able to disrupt the β-lactam ring before the antibiotic attaches to the penicillin-binding protein site, the antibacterial activity is lost. Once the penicillin has bound to the penicillin-binding protein, however, inhibition of membrane synthesis occurs and the penicillin is able to express its activity. Most of the penicillin can penetrate and bind to the penicillin-binding pro-

Table I. Classification of β-lactamases

Transmission	*Major substrate*	*Inhibitor*	*Bacterial source*	*Distribution*
Chromosome II	Penicillin	Isoxazolyl penicillins Clavulanic acid Sulbactam	*S. aureus* *Proteus* sp. *Pseudomonas* sp.	Major Minor Minor
Chromosome I	Cephalosporins	Isoxazolyl penicillins Carbenicillin	Enterobacteriaciae	Major
Chromosome IV	Penicillin and cephalosporins	Clavulanic acid Sulbactam	*E. coli* *Klebsiella* *Enterobacter* sp.	Major
Plasmid III, V	Penicillin and cephalosporins	Isoxazolyl penicillins Clavulanic acid Sulbactam	Enterobactriaciae	Major
Plasmid VI	Cephalosporin and penicillin	Isoxazolyl penicillin Clavulanic acid Sulbactam	*Bacteroides*	Major

Modified from Richmond and Sykes[9] and Sykes and Matthew.[10]

Table II. Examples of cephalosporin resistance to β-lactamases

		Relative hydrolysis rate				
β-Lactamase	*Bacteria*	*Cephalothin*	*Cefamandole*	*Cefotaxime*	*Moxalactam*	*Cefoxitin*
I	*Serratia*	273	7.7	0.5	0	0
I	*Proteus*	164	4.1	0	0	0
I	*Proteus*	234	187	21	0	0
I	*Pseudomonas*	351	4.2	0	0	0
III	*E. coli*	28	61	0	0	0
III	*Klebsiella*	24	59	0.5	0	0
IV	*Klebsiella*	20	27	0.5	0	0

Modified from Vuye.[11]

teins when small concentrations of enzyme are present in the periplasmic space. However, increased concentrations of enzyme can result if the antibiotic itself induces further production of β-lactamase. Some of the β-lactam antibiotics have the unfortunate property of inducing the production of β-lactamase in a number of organisms. A high level of β-lactamase allows the enzyme to destroy the antibiotic before attachment to penicillin-binding protein can occur (Fig. 3). It should be mentioned that, although penicillin is usually destroyed, in a newly described mechanism β-lactamase can also bind to the antibiotic without destruction of the penicillin itself.[8]

Several organisms have developed a high level of resistance to β-lactam antibiotics through the production of β-lactamase. Highly resistant gram-positive organisms include *S. aureus* and more recently, *Staphylococcus epidermidis.* Highly resistant gram-negative organisms include *Pseudomonas, Klebsiella,* and *Enterobacter* and *Bacteroides* sp., most frequently *B. fragilis, Bacteroides bivius,* and *Bacteroides disiens.* Both *H. influenzae* and, more recently, *Neisseria gonorrhoeae* have also developed the ability to produce β-lactamase. A wide variety of other species produce β-lactamase less commonly.

There are other, less important mechanisms that do not involve β-lactamase in which the activity of β-lactam antibiotics is reduced. A loss of activity occurring from reduced entry of the antibiotic into the microorganism is recognized to be particularly important for gram-negative bacteria. Gram-negative organisms have a more complex outer membrane than gram-positive organisms. The phospholipid in the outer membrane of gram-negative bacteria acts as a hydrophobic barrier that reduces the penetration of β-lactam antibiotics through the outer membrane pores (Fig. 1). Alterations of the penicillin-binding proteins that prevent penicillin attachment have also been proposed as a mechanism of reducing the action of penicillin but there is limited evidence for this hypothesis at present.

Classification of β-lactamases

Various β-lactamase classification systems have been proposed to assist and clarify enzyme research. To date, β-lactamases have been classified by substrate and inhibitor profiles, isoelectric point focusing, immunogenic activity, molecular weight, and genetic origin. The Richmond-Sykes classification system[9] originally described β-lactamases on the basis of substrate profiles, but this system was later modified by Sykes and Matthew[10] to combine these profiles with genetic control (Table I). β-Lactamase production can be either chromosomally or plasmid mediated.

Chromosomally mediated β-lactamases occur in most

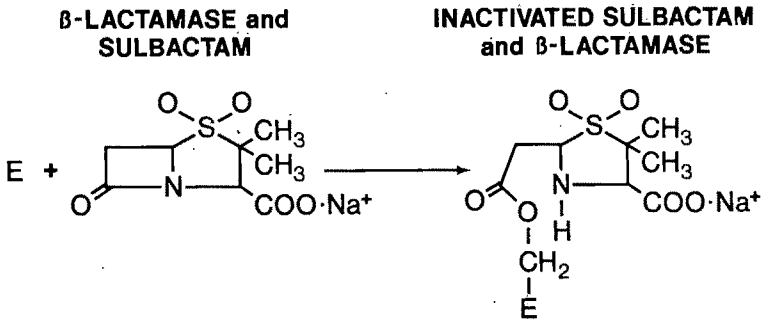


Fig. 4. Interaction of sulbactam, an irreversible β-lactamase inhibitor, with β-lactamases showing lethal action of sulbactam preventing regeneration of the enzyme and further hydrolysis.

organisms. The level of enzyme produced by chromosomal control is quite variable and ranges from barely detectable to high even in the absence of antibiotics. Increased β-lactamase production can occur either through spontaneous chromosomal mutation or by enzyme induction, a phenomenon that is increased by the presence of antibiotics. Penicillinase (Type II), cephalosporinase (Type I), and enzymes that hydrolyze both penicillin and cephalosporin (Type IV) are produced by chromosomally mediated genetic control. Type II penicillinase is present in *S. aureus, Proteus* sp. and *Pseudomonas* sp. It is inhibited by isoxazolyl penicillins, such as cloxacillin, and by β-lactamase inhibitors discussed below, sulbactam and clavulanic acid (Table I). Type I cephalosporinases are found in Enterobacteriaceae and they are inhibited by isoxazolyl penicillins and carbenicillin but not by clavulanic acid. Type IV β-lactamases that equally hydrolyze penicillins and cephalosporins are present in certain Enterobacteriaceae and they are inhibited by sulbactam and clavulanic acid.

Plasmids are extrachromosomal pieces of deoxyribonucleic acid that can be carried by bacteria. The extra genetic material is commonly transferred from one bacterium to another. Genetic information in the transferred plasma can be expressed if the material is incorporated into the host cell chromosome. If the plasmid deoxyribonucleic acid carries a code for antibiotic resistance (R plasmid), the host bacteria will develop resistance to that antibiotic or antibiotics. Plasmid-mediated resistance is of greater importance than chromosomally mediated resistance since it is more common in nature and it often confers resistance to multiple antibiotics at one time. Two categories of R-plasmid–mediated β-lactamases exist. One includes Type III β-lactamases that are active against both penicillins and cephalosporins. These enzymes are the most important of the β-lactamases because they are the most common. They are present in virtually all Enterobacteriaceae. Of the more than 10 Type III β-lactamases, the single most ubiquitous is present in 77% of Enterobacteriaceae. This enzyme is inhibited by cloxacillin, clavulanic acid, and sulbactam (Table I). Type V β-lactamase is also plasmid mediated with a profile similar to that of Type III. Type V is produced by *E. coli* and *Pseudomonas* sp. Type VI β-lactamase is produced by *Bacteroides*. The activity of this enzyme is inhibited by the isoxazolyl penicillins, clavulanic acid, and sulbactam (Table I).

Methods to reduce antibiotic resistance

There are several mechanisms to reduce or reverse antibiotic resistance. One successful method has been the formulation of β-lactamase–stable antibiotics. Broad-spectrum penicillins (ampicillin, amoxicillin, azlocillin, carbenicillin, mezlocillin, piperacillin, and ticarcillin) and the extended-spectrum cephalosporins (cefoxitin, cefamandole, moxalactam, cefotaxime, cefoperazone, cefotetan, etc.) are able to resist the action of one or more β-lactamases. The β-lactam rings of the broad-spectrum penicillins and extended-spectrum cephalosporins are hydrolyzed at a much lower rate than penicillin G or cephalothin. An example of the slower rate of hydrolysis of cefamandole, cefotaxime, moxalactam, and cefoxitin compared with that of cephalothin is provided in Table II.[11] The resistance to β-lactamase hydrolysis allows these extended-spectrum β-lactam antibiotics to maintain their antimicrobial activity despite the presence of enzymes.

A new method to reduce antibiotic resistance involves the combination of a β-lactamase inhibitor with a β-lactam antibiotic. There are several types of β-lactamase inhibitors. Sulbactam and clavulanic acid are two β-lactamase inhibitors that are termed suicide inhibitors because they irreversibly inactivate β-lactamases.[12] An example of the sulbactam reaction with β-lactamase *(E)* is provided in Fig. 4. A temporary, inactive β-lactamase–sulbactam complex is formed before the production of a stable, inactivated β-lactamase–sulbactam complex. The irreversible binding of these compounds to the active site of the β-lactamase inhibits the enzyme. Both the inhibitor and the enzyme are then irreversibly destroyed. The inhibitors themselves have virtually no inherent antibacterial activity. Therefore β-lactamase inhibitors are combined with a β-lactam antibiotic. The inhibitor destroys the β-lactamase, effectively restoring the antimicrobial activity of the antibiotic, which would

Table III. Minimum inhibitory concentration of ampicillin alone and combined with sulbactam or clavulanic acid

Organism	Geometric minimum inhibitory concentration (μg/ml)		
	Ampicillin alone	*Ampicillin with sulbactam, 5 μg/ml*	*Ampicillin with clavulanic acid, 5 μg/ml*
S. aureus	296	1.3	0.6
N. gonorrhoeae	40	2.2*	0.22*
E. coli	>2000	500	12
H. influenzae	>320	3.5	0.31
Klebsiella	500	7	1.7
Klebsiella	>2000	>500	15.6
B. fragilis	>80	62*	5.5*
B. fragilis	38	1.1*	0.8*

Modified from Hunter and Webb.[13]
*1 μl/ml.

Table IV. β-Lactamase inhibitors reduce minimum inhibitory concentration levels

	90% Minimum inhibitory concentration (μg/ml)				
	Ampicillin	*Ampicillin-sulbactam*	*Chloramphenicol*	*Clindamycin*	*Metronidazole*
B. fragilis group (n = 70)	256	8	16	256	4
Pigmented *Bacteroides* group (n = 22)	16	1	8	0.125	2
Other *Bacteroides* spp. (n = 46)	32	4	8	2	4

Modified from Wexler et al.[4]

otherwise be impaired by the enzyme. The result is a combination with greater antibacterial activity than that of the antibiotic alone. This increase in antibacterial activity varies, depending on the antibiotic and the inhibitor.

The role of these inhibitors in combination with a β-lactam antibiotic has been investigated in inhibition studies, in vitro susceptibility studies, and in vivo. Table III shows an example of the reduction of the minimum inhibitory concentration needed to inhibit organisms when sulbactam or clavulanic acid is combined with ampicillin. The minimum inhibitory concentration of various organisms to ampicillin alone is consistently higher (the antibiotic is less active) than the minimum inhibitory concentration of the organism to the combination of ampicillin-sulbactam or ampicillin–clavulanic acid. The addition of sulbactam or clavulanic acid to ampicillin increased the activity of that antibiotic.[13]

This is demonstrated in Table IV, where the 90% minimum inhibitory concentration of three different *Bacteroides* species is compared for ampicillin, chloramphenicol, clindamycin, metronidazole, and the combination of ampicillin-sulbactam. A total of 272 strains of anaerobic bacteria were tested.[14] Ampicillin-sulbactam was significantly more effective against *B. fragilis* and the other *Bacteroides* sp. than ampicillin alone. While the addition of β-lactamase inhibitors increases the activity of ampicillin, amoxicillin, ticarcillin, or cephalothin, β-lactamase inhibitors do not enhance the activity of all β-lactam antibiotics. The addition of β-lactamase inhibitors does little to enhance the activity of the newer, β-lactamase–stable antibiotics cefamandole, cefotaxime, moxalactam, or cefoxitin.[15]

Frequency of β-lactamase–producing bacteria in a review of obstetric-gynecologic infection

Thirty-six reports of infections in obstetrics and gynecology published between 1973 and 1986[16-51] were selected. Also included were 84 patients with postpartum endometritis at the University of Washington, in whom cultures were obtained as previously described[50] but the results have not yet been reported. In all reports, modern culture methods were used to recover organisms from infected areas. Enough patients had to be enrolled to provide a valid sample and the cultures had to be obtained from sites that are considered to be sterile in the uninfected patient. Organisms were isolated from the endometrium of women with postpartum endometritis[19-24, 26, 27, 32, 33, 40, 49, 50]; the cul-de-sac, abdomen, or tubes from women with acute pelvic inflammatory disease[43-48]; blood from patients with a wide variety of infections[17, 28-30, 35, 40, 41]; and wound and abscesses of women who had gynecologic operation.[31, 36] A final category in which the various infected sites could not be separated was also included (mixed infection).[16, 18, 25, 34, 37, 39, 42]

The isolates reported in these studies were coded into a software program in which 29 individual aerobic spe-

Table V. Percent of organisms from obstetric and gynecologic infections that produce β-lactamase

	β-Lactamase	Total (n = 2687*) (%)	Source of recovery				
			Endometrium (n = 1170) (%)	Tube (n = 327) (%)	Blood (n = 581) (%)	Gynecologic (n = 43) (%)	Mixed (n = 566) (%)
Aerobes							
Gram-positive							
S. aureus	+	2	2	<1	3	1	1
S. epidermidis	±	5	6	2	2	6	6
Streptococci, other	−	28	30	24	32	37	26
Gram-negative							
Enterobacteriaciae	+	13	14	6	26	30	13
Gonococci, *G. vaginalis*	−	5	2	11	4	0	9
Anaerobes							
Gram-negative							
Bacteroides	+	7	9	7	3	0	7
Bacteroides	±	10	9	8	9	5	13
Other anaerobes	−	5	6	5	1	3	3
Gram-positive	−	24	22	37	20	17	22
Total No. of isolates		6637	3377	964	419	78	1799

Data compiled from 36 studies published from 1973 to 1985.[16-51]
*n = Number of patients.

Table VI. Number of β-lactamase–producing organisms recovered from obstetric and gynecologic infections

β-Lactamase	Total	Source of recovery				
		Endometrium	Tube	Blood	Gynecologic	Mixed
+	1483	817	125	134	25	382
±	1008	512	96	46	9	345
−	4146	2048	743	239	44	1072
Total	3367	3377	964	419	78	1799

Data compiled from 36 studies published from 1973 to 1985.

cies, 18 individual anaerobic species, genital mycoplasmas, *Chlamydia trachomatis, Trichomonas vaginalis,* and *Candida* sp. were recorded. The bacterial species were then further divided into those isolates highly likely to produce β-lactamase (+), those isolates that have variable β-lactamase activity (±), and isolates that have a low prevalence of β-lactamase activity (−). It is emphasized that actual β-lactamase production was not reported in any of these studies and an estimate of the prevalence of β-lactamase activity was derived from in vitro data. No estimate was attempted of the concentration of β-lactamase that might be expected from the organisms. The organisms were then stratified according to expected high, variable, and low β-lactamase activity as shown in Table V. Infections occurred in a total of 2687 individuals in these 36 studies. A total of 6637 bacterial species were recovered at a rate of 2.5 organisms per patient.

A consistent prevalence of organisms in the three β-lactamase categories was demonstrated by the wide variety of obstetric and gynecologic infections listed. Facultative and anerobic gram-positive isolates and anaerobic gram-negative isolates in particular were recovered at similar rates in all of the infected sites. Enterobacteriaceae were less commonly isolated from the tubes–abdomen–cul-de-sac of women with acute pelvic inflammatory disease than from other infections. Enterobacteriaceae were also more frequently reported from the blood or abscesses than from other sites.

S. aureus, a frequent β-lactamase producer, accounted for 2% of the total isolates. *S. epidermidis,* a variable β-lactamase producer, accounted for 5% of isolates. Other facultative streptococci and other gram-positive organisms accounted for approximately 28% of total isolates. As mentioned, the proportion of isolates in these categories was consistent between infected sites. Enterobacteriaceae (in order of their frequency: *E. coli, Proteus mirabilis, Klebsiella* sp., *Enterobacter* sp., and *Citrobacter* sp.) and *Pseudomonas* sp. accounted for 13% of all isolates. Virtually all Enterobacteriaceae produce β-lactamase. *N. gonorrhoeae* and *Gardnerella vaginalis* each accounted for about 2.5% of the total isolates. These two organisms only infrequently produce β-lactamase.

Two frequent β-lactamase–producing gram-negative anaerobic bacteria, *B. bivius* (3.9%) and *B. fragilis* (3.6%), together were present in more than 7% of the total organisms isolated. Other *Bacteroides* sp., including *Bacteroides melaninogenicus,* that variably produce β-lactamase were found in 10% of the total isolates. Anaerobes that infrequently produce β-lactamase accounted for the remaining 29% of isolates.

In a summary of these findings, organisms expected to frequently produce β-lactamase were present among 1483 (22%) of the total 6637 isolates obtained from obstetric-gynecologic infections. An additional 15% of the total organisms isolated would be expected to variably produce β-lactamase. The proportion of potential β-lactamase–producing organisms showed only minimal variability among the various infected sites (Table VI). Although a mean of 2.5 organisms were isolated per patient, most of the patients would be expected to have only one of the β-lactamase producers. The actual number of patients who had more than one β-lactamase producer could not be determined from the data provided in these studies. From these data, it can be estimated that 20% to 30% of the obstetric and gynecologic infections contained organisms that produce β-lactamase.

The high proportion of β-lactamase–producing organisms in obstetric-gynecologic infections can have several consequences. One is the immediate resistance of the infecting organism to β-lactam antibiotics. The effectiveness of β-lactamase–stable antibiotics in the treatment of obstetric-gynecologic infections has become widely recognized. The use of new β-lactamase inhibitors such as sulbactam or clavulanic acid in combination with β-lactam antibiotics should also be useful in providing further resistance against β-lactamase and improving treatment results. Further experience with these new combinations is necessary.

The recovery of certain, less virulent β-lactamase–producing organisms such as *S. epidermidis* from infected upper genital tract sites has often not been considered particularly significant. However, the production of β-lactamase by one organism may potentially reduce the effective local β-lactam antibiotic level enough to influence successful therapy. In the throat, β-lactamase production by normal mouth flora reduces the rate of successful group A streptococci eradication by penicillin.[52] The failure of penicillin to consistently eliminate *N. gonorrhoeae* from the rectum has been attributed to the production of β-lactamase by rectal flora. Thus β-lactamase production by organisms of seemingly low virulence in the upper genital tract could theoretically be a cause of failure of antibiotic therapy to eradicate otherwise sensitive organisms from the upper tract. In addition, β-lactamase production by normal vaginal flora has not been studied, but it could be an explanation for the inability of ampicillin to eradicate group B streptococci from lower genital tract sites. Another possibility, particularly after antibiotic prophylaxis, is that vaginal β-lactamase production contributes to the selection of a wide variety of organisms, including those that do not produce β-lactamase. The persistence of one virulent organism in the vagina because of protection from another nonvirulent but β-lactamase–producing organism could contribute flora that subsequently causes upper tract infection. This circumstance would be particularly likely after cesarean section or prophylaxis associated with gynecologic operations.

An increase in the number of β-lactamase–producing organisms has been noted among women who have failure of therapy, and at least one fourth of patients can be expected to have infections that require treatment with antibiotics that are resistant to β-lactamases.[24, 35] Many obstetric-gynecologic infections involve β-lactamase–producing organisms. The use of newer broad-spectrum penicillins and cephalosporins has resulted in a higher cure rate compared with that of antibiotic regimens that do not contain β-lactamase–stable agents.[55] The combination therapy with β-lactamase inhibitors and β-lactam antibiotics should also result in high cure rates. It is now necessary to initiate treatment of most obstetric and gynecologic infections with either β-lactamase–stable antibiotics or the combination of β-lactamase inhibitors and a β-lactam antibiotic.

REFERENCES

1. Kirby WMM. Extraction of a highly potent inactivator from penicillin-resistant staphylococci. Science 1944;99: 452.
2. Finland M. Emergence of antibiotic resistance in hospitals 1935-1975. Rev Infect Dis 1979;1:4.
3. Neu HC. The emergence of bacterial resistance and its influence on empiric therapy. Rev Infect Dis 1983;5:9.
4. Matthew M. Plasmid-mediated β-lactamases of gram-negative bacteria: properties and distribution. J Antimicrob Chemother 1979;5:349.
5. Sabath LD. Mechanisms of resistance of beta-lactam antibiotics in strains of *Staphylococcus aureus.* Ann Intern Med 1982;97:339.
6. Moss J, Wyatt G, Christopherson D, Routh S. A prospective drug utilization review on the prescribing of oral and parenteral cephalosporins. Hosp Formulary 1982;17: 1589.
7. Ogawara H. Antibiotic resistance in pathogenic and producing bacteria with special reference to beta-lactam antibiotics. Microbiol Rev 1981;45:591.
8. Sanders CC, Sanders WE. Emergence of resistance during therapy with the newer β-lactam antibiotics: role of inducible β-lactamases and implications for the future. Rev Infect Dis 1983;5:639.
9. Richmond MH, Sykes RB. The β-lactamases of gram-negative bacteria and their possible physiological role. Adv Microb Physiol 1973;9:31.
10. Sykes RB, Matthew M. The beta-lactamases of gram-negative bacteria and their role in resistance to beta-lactam antibiotics. J Antimicrob Chemother 1976;2:115.
11. Vuye A. Comparative stability of first, second, and third generation cephalosporins and N-formimidoyl thiena-

mycin against gram-negative beta-lactamases. Arzneimittelforschung 1983;33:1236.
12. Labia R, Lelievre V, Peduzzi J. Inhibition kinetics of three R-factor-mediated beta-lactamase by a new beta-lactam sulfone (CP 45899). Biochim Biophys Acta 1980;611: 351.
13. Hunter PA, Webb JR. In: Nelson JD, Grassi C, eds. Current chemotherapy and infectious disease: proceeding of the 11th International Congress of Chemotherapy and the 19th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society of Microbiology, 1980:340.
14. Wexler HM, Harris B, Carter WT, Finegold SM. In vitro efficacy of sulbactam combined with ampicillin against anaerobic bacteria. Antimicrob Agents Chemother 1985; 27:876.
15. Lamothe F, Auger F, Lacroix J-M. Effect of clavulanic acid on the activities of ten beta-lactam agents against members of the *Bacteroides fragilis* group. Antimicrob Agents Chemother 1984;25:662.
16. Sweet RL, Ledger WJ. Puerperal infectious morbidity: a two-year review. AM J OBSTET GYNECOL 1977;117: 1093.
17. Cunningham FG, Hauth JC, Strong JD, Kappus SS. Infectious morbidity following cesarean section: comparison of two treatment regimens. Obstet Gynecol 1978;52: 656.
18. Gilstrap LC III, Maier RC, Gibbs RS, Connor KD, St Clair PJ. Piperacillin versus clindamycin plus gentamicin for pelvic infections. Obstet Gynecol 1984;64:762.
19. Gibbs RS, Blanco JD, Castaneda YS, St Clair PJ. A double-blind, randomized comparison of clindamycin-gentamicin versus cefamandole for treatment of post–cesarean section endomyometritis. AM J OBSTET GYNECOL 1982;144: 261.
20. Ledger WJ, Gee CL, Pollin PA, Lewis WP, Sutter VL, Finegold SM. A new approach to patients with suspected anaerobic postpartum pelvic infections: transabdominal uterine aspiration for culture and metronidazole for treatment. AM J OBSTET GYNECOL 1976;126:1.
21. Gibbs RS, Blanco JD, Duff P, Castaneda YS, St Clair PH. A double-blind, randomized comparison of moxalactam versus clindamycin-gentamicin in treatment of endomyometritis after cesarean section delivery. AM J OBSTET GYNECOL 1983;146:769.
22. Gilstrap LC III, Cunningham FG. The bacterial pathogenesis of infection following cesarean section. Obstet Gynecol 1979;53:545.
23. Platt LD, Yonekura ML, Ledger WJ. The role of anaerobic bacteria in postpartum endomyometritis. AM J OBSTET GYNECOL 1979;135:814.
24. Gibbs RS, Jones PM, Wilder CJ. Antibiotic therapy of endometritis following cesarean section: treatment successes and failures. Obstet Gynecol 1978;52:31.
25. Sweet RL, Robbie MO, Ohm-Smith M, Hadley WK. Comparative study of piperacillin versus cefoxitin in the treatment of obstetric and gynecologic infections. AM J OBSTET GYNECOL 1983;145:342.
26. Burkman RT, Atienza MF, King TM. Culture and treatment results in endometritis following elective abortion. AM J OBSTET GYNECOL 1977;128:556.
27. Gibbs RS, O'Dell TN, MacGregor RR, Schwarz RH, Morton H. Puerperal endometritis: a prospective microbiologic study. AM J OBSTET GYNECOL 1975;121:919.
28. Monif GRG, Baer H. Polymicrobial bacteremia in obstetric patients. Obstet Gynecol 1976;48:167.
29. Ledger WJ, Norman M, Gee C, Lewis W. Bacteremia on an obstetric-gynecologic service. AM J OBSTET GYNECOL 1975;121:205.
30. Bryan CS, Reynolds KL, Moore EE. Bacteremia in obstetrics and gynecology. Obstet Gynecol 1984;64:155.
31. Glover MW, van Nagell JR. The effect of prophylactic ampicillin on pelvic infection following vaginal hysterectomy. AM J OBSTET GYNECOL 1976;126:385.
32. Hemsell DL, Cunningham FG, DePalma RT, Nobles BJ, Heard M, Hemsell PG. Cefotaxime sodium therapy for endomyometritis following cesarean section: dose-finding and comparative studies. Obstet Gynecol 1983;62:489.
33. Hagglund L, Christensen KK, Christensen P, Kamme C. Risk factors in cesarean section infection. Obstet Gynecol 1983;62:145.
34. Gall SA, Kohan AP, Ayers OM, Hughes CE, Addison WA, Hill BG. Intravenous metronidazole or clindamycin with tobramycin for therapy of pelvic infections. Obstet Gynecol 1981;57:51.
35. diZerega GS, Yonekura ML, Keegan K, Roy S, Nakamura R, Ledger W. Bacteremia in post-cesarean section endomyometritis: differential response to therapy. Obstet Gynecol 1980;55:587.
36. Hemsell DL, Reisch J, Nobles B, Hemsell PG. Prevention of major infection after elective abdominal hysterectomy: individual determination required. AM J OBSTET GYNECOL 1983;147:520.
37. Sweet RL, Ohm-Smith M, Landers DV, Robbie MO. Moxalactam versus clindamycin plus tobramycin in the treatment of obstetric and gynecologic infections. AM J OBSTET GYNECOL 1985;152:808.
38. Sorrell TC, Marshall JR, Yoshimori R, Chow AW. Antimicrobial therapy of postpartum endomyometritis. II. Prospective, randomized trial of mezlocillin versus ampicillin. AM J OBSTET GYNECOL 1981;141:246.
39. Gall SA, Hill GB. High-dose cefamandole therapy in obstetric and gynecologic infections. AM J OBSTET GYNECOL 1980;137:914.
40. Gibbs RS, Huff RW. Cefamandole therapy of endomyometritis following cesarean section. AM J OBSTET GYNECOL 1980;136:32.
41. Lamey JR, Eschenbach DA, Mitchell SH, Blumhagen JM, Foy JM, Kenny GE. Isolation of mycoplasmas and bacteria from the blood of postpartum women. AM J OBSTET GYNECOL 1982;143:104.
42. Duff P, Keiser JF. A comparative study of two antibiotic regimens for the treatment of operative site infections. AM J OBSTET GYNECOL 1982;142:996.
43. Monif GR. Significance of polymicrobial bacterial superinfection in the therapy of gonococcal endometritis-salpingitis-peritonitis. Obstet Gynecol 1980;55:154S.
44. Eschenbach DA, Buchanan TM, Pollock HM, et al. Polymicrobial etiology of acute pelvic inflammatory disease. N Engl J Med 1975;293:166.
45. Cunningham FG, Hauth JC, Gilstrap LC, Herbert WNP, Kappus SS. The bacterial pathogenesis of acute pelvic inflammatory disease. Obstet Gynecol 1978;52:161.
46. Chow AW, Patten V, Marshall JR. Bacteriology of active pelvic inflammatory disease: suboptimal survival of *Neisseria gonorrhoeae* in a nonbuffered transport system. AM J OBSTET GYNECOL 1979;133:362.
47. Sweet RL, Draper DL, Schachter J, James J, Hadley WK, Brooks GF. Microbiology and pathogeneis of acute salpingitis as determined by laparoscopy: what is the appropriate site to sample? AM J OBSTET GYNECOL 1980; 138:985.
48. Thompson SE, Hager WD, Wong K-H, et al. The microbiology and therapy of acute pelvic inflammatory disease in hospitalized patients. AM J OBSTET GYNECOL 1980; 136:179.
49. Gibbs RS, Blanco JD, Castaneda YS, St Clair PJ. Therapy of obstetrical infections with moxalactam. Antimicrob Agents Chemother 1980;17:1004.
50. Blanco JD, Gibbs RS, Duff P, Castaneda YS, St Clair PJ. Randomized comparison of cefotaxime versus clindamycin-tobramycin in the treatment of obstetrical and gynecological infections. Antimicrob Agents Chemother 1983;24:500.
51. Brooks I. Role of beta-lactamase-producing bacteria in the failure of penicillin to eradicate Group D streptococci. Pediatr Infect Dis 1985;4:491.

Induction of β-lactamase enzymes: Clinical applications for the obstetric-gynecologic patient

David L. Hemsell, M.D.
Dallas, Texas

The emergence of bacteria resistant to antibiotics has resulted in intensive research for new and improved β-lactam antibiotics. Many improvements in antimicrobial agents are based on a knowledge of the mechanism responsible for resistance. This has led to the development of new extended-spectrum antibiotic compounds. However, several features have been noted since the development of extended-spectrum antibiotics, such as the rapid development of bacterial resistance, the induction of β-lactamase enzyme activity by these stable antibiotics, failure to detect induced enzyme activity and resistance in the laboratory, and β-lactam antagonism. The resistance of bacteria to antimicrobial agents has obvious impact on the selection of appropriate therapy against infections caused by these pathogens. Gram-negative anaerobic bacteria, such as *Bacteroides fragilis* and *Bacteroides bivius*, are organisms frequently recovered from women whose initial therapy for pelvic infection failed. The transfer of antimicrobial resistance in bacteria indicates that these organisms have a system for the spread of such resistance. Therefore determination of antimicrobial susceptibilities and prompt eradication of isolates from infected patients are necessary to delay the emergence of resistant organisms. (AM J OBSTET GYNECOL 1987;156:504-6.)

Key words: β-Lactam antibiotic, antibiotic resistance, induction, antagonism

Before the introduction of penicillin in 1941, Abraham and Chain[1] had reported the observation that an enzyme produced by *Escherichia coli* prevented the bactericidal activity of benzylpenicillin. There has been a rapid expansion of the β-lactam technology during the past 45 years. The intensive search for new and improved β-lactam antibiotics has arisen because of the development of resistance to antibiotics in the clinical setting.

Three variables impact on the ability of bacteria to develop antibiotic resistance, including alterations in the structure or affinity of penicillin-binding proteins, alterations to cell membrane permeability, and production of β-lactamase enzymes. Current drug research has been aimed at developing β-lactam antibiotics that are resistant to the principal means of antimicrobial inactivation by bacteria—the production of β-lactamase enzymes.

β-Lactamase enzyme production

The production of β-lactamase in bacteria may be mediated through extra chromosomal genetic information, termed R factors or plasmids, or it can be chromosomally mediated. Most β-lactamase production is plasmid mediated rather than chromosomally mediated.[2] There are, however, several bacteria that possess a chromosomally mediated ability to dramatically increase the production of β-lactamase after exposure to an inducer, the principal inducers being the β-lactam antibiotics.

β-Lactamase activity sites vary among the different bacteria. In gram-negative bacteria, the predominant β-lactamase activity occurs within the inner and outer cell wall membranes in the periplasmic space. For gram-positive bacteria such as *Staphylococcus aureus*, the β-lactamase exerts its activity extracellularly. Several β-lactamases have been identified in gram-negative aerobic bacteria. The Richmond and Sykes classification system[2] was developed to categorize these enzymes.

Class I enzymes are chromosomally mediated and have cephalosporins as their primary substrate. The genetic expression of these enzymes is that they are inducible. The clinical pathogens in which they are seen are the gram-negative aerobic pathogens *Enterobacter, Citrobacter, Serratia* and *Proteus* sp., and *Pseudomonas aeruginosa*.

Class II β-lactamases are clinically rare and have penicillins as their primary substrate. They are chromosomally mediated and constitutive, or produced at a constant rate.

The principal gram-negative β-lactamase enzyme observed in bacteria recovered from the obstetric and gynecologic patient population is in the Class III category. The substrates for these enzymes are both pen-

From the Department of Obstetrics and Gynecology, University of Texas Health Science Center.
Reprint requests: Dr. David L. Hemsell, University of Texas Health Science Center, Department of Obstetrics and Gynecology, G6216, 5323 Harry Hines Blvd., Dallas, TX 75235.

icillins and cephalosporins, and the enzyme is produced constitutively. These enzymes are plasmid mediated and account for approximately 85% of β-lactamases observed. The clinical pathogens secreting these enzymes are *Neisseria gonorrhoeae, Haemophilus influenzae, Pseudomonas aeruginosa,* and various species in the Enterobacteriaceae family.

Class IV β-lactamases are active against both penicillins and cephalosporins and are chromosomally mediated. They are constitutive and are seen uncommonly, primarily in *Klebsiella* sp.

Class V enzymes are effective against penicillins, cephalosporins, and isoxazolyl penicillins. This class constitutes the remaining 15% of the plasmid-mediated β-lactamase production. This enzyme is also constitutive and is found primarily in *Pseudomonas* sp. and in species of the Enterobacteriaceae family.

The β-lactamase secreted by gram-positive aerobic bacteria is effective primarily against penicillins and is inducible. It has been observed in both *Staphylococcus aureus* and *Staphylococcus epidermidis.*

β-Lactam antibiotics

Several of the β-lactam antibiotics, including the penicillin and ampicillin families and the first-generation cephalosporins, are generally susceptible to β-lactamase. The enzyme binds to and hydrolyzes the β-lactam ring, thus rendering the antimicrobial ineffective. The ineffective metabolite is released, and the β-lactamase that is still active inactivates another antimicrobial molecule.

Current research has been aimed at the development of antimicrobials that are β-lactamase stable. Some of these newer antibiotics include methicillin, nafcillin, the oxacillins, second- and third-generation cephalosporins, penems, and thienamycin. While it was believed that these newer antibiotics would resist enzymatic degradation, induction of β-lactamase enzyme activity by these enzyme-stable antibiotics has recently been observed.[3]

β-Lactamase induction

Recent studies have demonstrated that bacteria possessing inducible β-lactamases may develop resistance to a variety of β-lactam antibiotics. It appears that the enzyme induced in bacteria is either reversible, if the inducer is a β-lactam antibiotic, or irreversible, if overproduction of the enzyme occurs as a result of mutation. The β-lactamase enzyme, once induced, renders the strain resistant to many β-lactam antibiotics, including the newer, more stable compounds that are not hydrolyzed by the enzymes. Enzyme-antimicrobial binding does occur, but it is nonhydrolytic, which apparently acts as a stimulus to dramatically increase the production of β-lactamase enzyme. When mutation occurs, resistance to a multiplicity of antibiotics in specific organisms or bacteria is observed.

Enzyme inducer activity

Primary research has involved exposure of certain bacteria to various antibiotics in order to establish an inducer profile. One such study using *Enterobacter cloacae* demonstrated that low inducers, that is, the antibiotics that do not stimulate bacteria to increase their production of β-lactamase enzyme, are methicillin, piperacillin, and cefoperazone.[4] Intermediate inducers observed were carbenicillin, cefotaxime, and cefuroxime and high inducers included cefazolin, cephalothin, cefamandole, cefoxitin, moxalactam, and clavulanic acid.

These results identify a problem with the enzyme-induction concept since, theoretically, the antibiotic that is resistant to the hydrolytic activity of the β-lactamase should be the inducer. Contrariwise, those that serve as a good substrate for hydrolytic activity and are destroyed should not be high inducers of enzyme production. Additionally, this phenomenon may be strain-dependent. In another study that added further confounding data, induction of β-lactamase was studied in an *E. cloacae* strain isolated from a clinical specimen.[5] Large amounts of the enzyme were induced in the presence of β-lactams. In this study, cefoxitin and mezlocillin were low inducers, while penicillin and moxalactam were high inducers. Other cyclic structures may also induce enzyme activity; these include folic acid, hemin, trypticase soy broth, human serum, pleural fluid, cerebrospinal fluid, and Schaedler's broth. Cefoxitin was most potent as an inducer in trypticase soy broth.

The importance of the induction of β-lactamase enzymes in clinical medicine is unknown. This phenomenon has been observed in the laboratory in bacteria recovered from patients whose antibiotic therapy was successful or unsuccessful. The identification of this phenomenon does not therefore imply clinical relevance.

Detection of induced enzyme activity is relatively difficult to identify in the laboratory. Microdilution and disk diffusion techniques do not identify induction until after it has become clinically evident. The clinical manifestation of enzyme induction is either a failure to respond to therapy or the development of a superinfection. Enzyme induction in the laboratory, however, can be confirmed with the macrodilution technique and use of an inoculum of $\geq 10^5$ cfu/ml of organisms.

Another distressing phenomenon that has been observed is that of β-lactam antagonism. The potential for antagonism between some of the newer β-lactam antibiotics lies in the ability of the enzyme-stable antibiotics to act as inducers of β-lactamase. The drug-

induced enzyme hydrolyzes or creates a barrier against access to target proteins.

Antagonism has been observed specifically with cefoxitin both in vivo and in vitro.[6] It is seen when cefoxitin and other β-lactam antibiotics are combined and administered for an infection caused by bacteria with the inducible enzyme system. It is also seen clinically when a β-lactam antibiotic is given as pretreatment or prophylaxis or when treatment is given with subinhibitory dosing. Research in this field indicates that it is the responsibility of the microbiologist to identify the bacteria mentioned above, bacteria that have the potential for inducing the production of β-lactamase, and to inform the physician so that he or she can find alternative therapy to the β-lactam antimicrobials should there be evidence of clinical failure.

Consequences for the obstetric-gynecologic patient

Most obstetric and gynecologic patients do not develop pelvic infections caused by organisms in which β-lactamase enzyme induction is a problem. However, there does seem to be increasing antibiotic resistance because of several factors, including prolonged antimicrobial prophylaxis, that may accelerate the problem of altered resistance patterns, in addition to induced resistance.

REFERENCES

1. Abraham EP, Chain E. An enzyme from bacteria able to destroy penicillin. Nature 1940;146:837.
2. Richmond MH, Sykes RB. The beta-lactamases of gram-negative bacteria and their possible physiological role. Adv Microb Physiol 1973;9:31.
3. Sanders CC. Novel resistance selected by the new expanded-spectrum cephalosporins: a concern. J Infect Dis 1983;147:585.
4. Minami S, Yotsuji A, Inoue M, Mitsuhashi S. Induction of β-lactamase by various β-lactam antibiotics in *Enterobacter cloacae*. Antimicrob Agents Chemother 1980;18:382.
5. Cullman W, Dalhoff A, Dick W. Nonspecific inductor of β-lactamase in *Enterobacter cloacae*. J Gen Microbiol 1984; 130:1781.
6. Sanders CC, Sanders WE, Goering RV. In vitro antagonism of beta-lactam antibiotics by cefoxitin. Antimicrob Agents Chemother 1982;21:968.

Ampicillin/sulbactam versus metronidazole-gentamicin in the treatment of soft tissue pelvic infections

William R. Crombleholme, M.D., Marilyn Ohm-Smith, Marilyn O. Robbie, Vicki DeKay, and Richard L. Sweet, M.D.

San Francisco, California

The clinical efficacy and safety of ampicillin/sulbactam versus metronidazole-gentamicin were evaluated in a comparative, randomized, prospective study. Forty-four patients were enrolled: 22 received the ampicillin/sulbactam regimen, and 22 received the metronidazole-gentamicin combination. There were 33 cases of severe acute pelvic inflammatory disease, two tuboovarian abscesses, five cases of endomyometritis, and two cases of posthysterectomy pelvic cellulitis. Aerobic and anaerobic cultures from the infection sites yielded 447 microorganisms from 44 patients (an average of 10 bacteria per infection; 6.4 anaerobes and 3.7 aerobes). The most frequent isolates were *Bacteroides sp.*, 54; *Bacteroides bivius*, 17; black-pigmented *Bacteroides*, 12; *Bacteroides disiens*, 11; *Fusobacterium*, 13; *Peptostreptococcus anaerobius*, 24; *Peptostreptococcus asaccharolyticus*, 21; anaerobic gram-positive cocci, 34; *Gardnerella vaginalis*, 29; *Neisseria gonorrhoeae*, 17; α-hemolytic streptococci, 15; and *Escherichia coli*, five. Clinical cure was noted in 19 of 20 patients treated with ampicillin/sulbactam and 18 of 21 patients treated with metronidazole-gentamicin. One treatment failure occurred in the ampicillin/sulbactam group in a patient who required antichlamydial therapy and had a complex left adnexal mass consistent with an abscess. The cases of metronidazole-gentamicin failure included two patients initially diagnosed as having tuboovarian abscesses who required a change in antibiotic therapy to control the infections. The third patient had postabortion endomyometritis that did not respond to metronidazole-gentamicin therapy within 48 hours, and required a change of medication. No adverse hematologic, renal, or hepatic effects were noted in either group of patients. (Am J Obstet Gynecol 1987;156:507-12.)

Key words: Pelvic infections, sulbactam, ampicillin, gentamicin, metronidazole

Although an array of antimicrobial agents are currently available today, soft tissue pelvic infections continue to be a major cause of morbidity in obstetric and gynecologic patients.[1] The most common soft tissue pelvic infections diagnosed in a gynecologic-obstetric service include posthysterectomy pelvic cellulitis, vaginal cuff abscess, pelvic abscess, pelvic inflammatory disease, tuboovarian abscess, post–cesarean section infection, endomyometritis, septic abortion, and septic pelvic thrombophlebitis.[2]

The pathogens responsible for soft tissue pelvic infections include a wide variety of aerobic and anaerobic organisms. The aerobic organisms include *Escherichia coli*, group B streptococci, and other facultative streptococci. The anaerobic organisms include: *Bacteroides bivius*, other *Bacteroides*, and *peptostreptococci*. Of the several underlying causes and characteristics of these infections, two are of particular importance. First, many of the organisms responsible for obstetric and gynecologic infections (with the exception of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and group A streptococci) arise from the normal flora of the lower genital tract. Second, many of these infections have a "polymicrobial etiology"[3,4]; that is, these infections are due to multiple organisms including mixtures of anaerobes and aerobic organisms or mixtures of anaerobic bacteria.

Study results accumulated during the last 5 years have underscored the importance of initial antimicrobial therapy aimed at eradicating resistant anaerobes, particularly *Bacteroides*.[2,5-7] Several studies have examined the effectiveness of single-agent and multiple-agent therapies including clindamycin-aminoglycoside, metronidazole-aminoglycoside, and, more recently, such combinations as clindamycin-aztreonam.[8,9] Several single-agent regimens have been shown prospectively to be fairly effective in treating mixed pelvic infections.[10-12] These agents include second- and third-generation cephalosporins, the extended-spectrum penicillins, and some of the newer cephalosporin-related compounds.

An alternative option to the treatment of mixed anaerobic-aerobic infections associated with resistant β-lactamase enzyme–producing bacteria has been suggested. This approach entails the use of a β-lactamase

From the Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, and San Francisco General Hospital.

Reprint requests: Dr. Richard L. Sweet, Department of Obstetrics and Gynecology, San Francisco General Hospital, 1001 Potrero Ave., San Francisco, CA 94110.

Table I. Patient population

	A/S (N = 22)	*M-G (N = 22)*
Age (yr)		
Range	19-47	18-72
Mean ± SD	27.7 ± 6.9	29.0 ± 12.1
Race		
White	7	9
Black	13	9
Hispanic	1	3
Asian	1	1
Gravidity		
Range	0-7	0-4
Mean ± SD	2.9 ± 2.1	1.9 ± 1.4
Parity		
Range	0-4	0-4
Mean ± SD	1.2 ± 1.2	0.9 ± 1.0
Temperature (°C)		
Range	37.0 - 40.1	36.8 - 39.7
Mean ± SD	38.1 ± 0.8	38.3 ± 0.7
White blood cell count (No. × 1000/mm³)		
Range	5.0 - 21.1	6.5 - 22.3
Mean ± SD	10.8 ± 4.2	13.0 ± 4.8
Duration of therapy (days)		
Range	3-7	2-11
Mean ± SD	5.2 ± 1.1	5.7 ± 1.9

enzyme inhibitor combined with an available β-lactam antimicrobial agent such as ampicillin, amoxicillin, or ticarcillin. The result is an enhanced spectrum of activity against β-lactamase enzyme–producing bacteria, including *E. coli, B. bivius,* and penicillinase-producing *N. gonorrhoeae.*

The randomized, prospective study reported herein was designed to evaluate the safety and efficacy of the combination therapy ampicillin/sulbactam as compared with metronidazole-gentamicin in the treatment of mixed aerobic and anaerobic soft tissue pelvic infections.

Material and methods

The study was conducted in hospitalized women 18 years of age or older, at San Francisco General Hospital, San Francisco, California, with a documented or clinical diagnosis of mixed aerobic-anaerobic pelvic infection. Excluded from the study were pregnant or lactating women; patients who were allergic to penicillins, cephalosporins, aminoglycosides, or metronidazole; those with impaired renal function (serum creatinine >1.8 mg/100 ml); those with a family history of glycogen storage disease or recurrent hypoglycemia; or those with a history of unstable cardiovascular, hepatic, renal, or neurological disease.

The patients underwent a series of pretreatment laboratory tests which served as baseline values and were repeated every 4 days. These tests included complete blood count with differential and platelet counts, total bilirubin, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, direct Coombs test, serum creatinine, urinalysis, serum pregnancy test by the β-subunit of human chorionic gonadotropin (on admission), and hepatitis B surface antigen (on admission). Blood specimens were taken and cultured for anaerobic and facultative organisms on admission before the start of antibiotic therapy. Cervical cultures were performed only for *N. gonorrhoeae* and *C. trachomatis.* Endometrial brush samples were taken with a double-lumen catheter and the specimens were cultured for *N. gonorrhoeae, C. trachomatis, Mycoplasma hominis,* and *Ureaplasma urealyticum,* as well as for anaerobic and facultative organisms by means of methods previously described.[14] Culdocentesis was performed to obtain peritoneal fluid in patients with posthysterectomy cuff cellulitis. Isolates were tested for β-lactamase production using discs (Cefinase, BBL, Cockeysville, Maryland).

Patients were then randomized prospectively to receive one of two antibiotic regimens. The ampicillin/sulbactam (A/S) group received ampicillin, 2 gm, and sulbactam, 1 gm, intravenously every 6 hours. The metronidazole-gentamicin (M-G) group received metronidazole, 15 mg/kg intravenously as a loading dose, then 7.5 mg/kg intravenously every 6 hours in combination with gentamicin, 1.5 mg/kg intravenously every 8 hours. Therapy continued until patients became afebrile and were without clinical signs of infection for 48 hours or until clinical judgment dictated cessation of therapy. Generally the antibiotics were administered for at least 5 days.

Forty-four patients were enrolled in the study, half in the A/S group and half in the M-G group. The A/S group included 14 patients with acute pelvic inflammatory disease, two with tuboovarian abscesses, four with endomyometritis, and two with posthysterectomy pelvic infections. The M-G group included 19 patients with pelvic inflammatory disease, one with tuboovarian abscess, one with endomyometritis, and one with posthysterectomy infection.

Patient population. The 44 patients in this study were representative of the general clinic patient population at San Francisco General Hospital (Table I). Demographically the patients in both groups were similar with respect to age, racial background, gravidity, and parity. Clinically the patients were also similar. The A/S group had a slightly lower mean white blood cell count, 10.8 compared with 13.0 in the M-G group. Duration of therapy was also similar in both groups, 5.2 versus 5.7 days.

Pretreatment cultures. The results of the direct plate and broth cultures were typical for what might be expected in patients with soft tissue pelvic infections, that is, a large number of mixed aerobic and anaerobic organisms, particularly *Bacteroides.* Table II shows the

Table II. Aerobic isolates from pretreatment cultures

Organism	A/S (N = 22)		M-G (N = 21)	
	No. of isolates from pretreatment cultures	*No. of isolates from primary plates*	*No. of isolates from pretreatment cultures*	*No. of isolates from primary plates*
Gram-positive cocci	30	18	21	9
Staphylococcus aureus	1	1	0	0
Coagulase-negative staphylococci	11	6	5	3
Group B streptococci	2	1	1	0
Group D streptococci, not enterococci	3	2	3	1
Enterococci	1	1	1	1
α-Hemolytic streptococci	7	4	8	2
Nonhemolytic streptococci, not group B or D	5	3	2	1
Streptococcus viridans	0	0	1	1
Gram-negative cocci	8	8	9	9
N. gonorrhoeae	8	8	9	9
Gram-positive rods	22	17	19	14
Lactobacilli	14	9	11	9
Diphtheroids	8	8	8	5
Gram-negative rods	26	25	30	26
Gardnerella vaginalis	14	13	15	13
Unidentified coryneform organisms	8	8	11	10
E. coli	3	3	2	1
P. mirabilis	0	0	1	1
Haemophilus influenzae	1	1	1	1

aerobic isolates from pretreatment cultures. The gram-positive organisms included primarily *Staphylococcus epidermidis* and hemolytic and nonhemolytic streptococci. *N. gonorrhoeae* was the most commonly encountered gram-negative organism. *Gardnerella vaginalis* was also frequently cultured, although mainly in primary plates rather than broth cultures. Table III lists the anaerobic isolates from the pretreatment cultures. The most common gram-positive organisms cultured were *peptostreptococci,* including *Peptostreptococcus asaccharolyticus, Peptostreptococcus anaerobius,* and *Peptostreptococcus tetradius.* The *Bacteroides* were the most common gram-negative anaerobes cultured. *B. bivius* was the most frequent isolate of the gram-negative anaerobes cultured. Also commonly seen were *Bacteroides disiens, Bacteroides capillosus,* and black-pigmented *Bacteroides.* Of particular interest is the finding in four patients of *Mobiluncus* sp. isolated from the upper genital tract. In addition, *M. hominis* was commonly isolated, while *Ureaplasma urealyticum* was not. *C. trachomatis* was also isolated from patients with pelvic inflammatory disease.

Results

Table IV shows the clinical response of those patients receiving the A/S regimen and those receiving the M-G regimen. One patient in the A/S group with a clinical diagnosis of pelvic inflammatory disease and a right adnexal mass was excluded. Laparoscopy revealed a right ovarian cyst, no visual evidence of infection, and essentially no growth from cervical and endometrial cultures. One patient in the M-G group with a clinical diagnosis of pelvic inflammatory disease was excluded; she left the hospital against medical advice on the third hospital day. Clinical cure was defined as a response to the antimicrobial combination alone without the need for surgical intervention or additional therapeutic agents. A/S proved to be as effective as M-G in treating mixed aerobic and anaerobic soft tissue pelvic infections in our groups of patients. In the 21 patients who completed treatment in the A/S group, clinical cure was demonstrated in 16 (76%) and in 16 of the 21 in the M-G group. Another three patients (14%) in the A/S group and two (9%) in the M-G group demonstrated clinical cure after doxycycline was added to the regimen to treat chlamydial infections. When these subsets are combined, the A/S group demonstrated a 90% cure rate compared with an 85% clinical cure rate in the M-G group.

One patient in the A/S group was considered to be a clinical failure because she developed an allergic reaction and had to stop taking the medication. There were no allergic reactions in the patients receiving M-G. One additional patient in the A/S group and three in the M-G group were considered to be treatment failures. The patient in the A/S group had acute pelvic inflammatory disease and did not respond to A/S treatment within 3 days. Results of a sonographic examination showed a complex cystic mass in the left adnexa, which may have been present at the time of admission but was obscured by peritoneal and pelvic tenderness. Antibiotic therapy was changed to tobramycin plus clindamycin and the patient responded. Among the three treatment failures in the M-G group, two patients had pelvic inflammatory disease and a tuboovarian abscess.

Table III. Anaerobic isolates from pretreatment group

Organism	A/S (N = 22)		M-G (N = 21)	
	No. of isolates from pretreatment group	*No. of isolates from primary plates*	*No. of isolates from pretreatment group*	*No. of isolates from primary plates*
Gram-negative rods	60	33	43	25
B. bivius	11	9	6	5
B. capillosus	4	1	2	0
B. disiens	5	4	6	5
B. fragilis group	2	0	1	0
Black-pigmented *Bacteroides*	8	4	4	4
Bacteroides sp.	30	15	24	11
Gram-negative rods	14	10	15	11
Fusobacterium sp.	6	4	7	5
Unidentified anaerobic gram-negative rods	5	4	7	5
Mobiluncus	3	2	1	1
Gram-positive rods	13	8	13	6
Lactobacillus sp.	6	4	3	1
Bifidobacterium sp.	1	0	2	0
Eubacterium sp.	0	0	1	0
Actinomyces sp.	1	1	0	0
Unidentified anaerobic gram-positive non-sporing rod	4	3	6	5
Clostridium innocuum	0	0	1	0
Clostridium perfringens	1	0	0	0
Gram-positive cocci	52	31	56	28
P. tetradius	7	4	6	3
P. asaccharolyticus	12	5	9	3
P. magnus	1	1	5	4
P. prevotii	4	3	6	2
P. anaerobius	14	10	10	6
Unidentified anaerobic gram-positive cocci	14	8	20	10
Gram-negative cocci	6	3	10	4
Veillonella parvula	1	0	4	1
Unidentified anaerobic gram-negative cocci	5	3	6	3
Other				
Mycoplasma sp.	5		3	
Ureaplasma urealyticum	2		0	
Chlamydia trachomatis	3		4	

Table IV. Clinical response with A/S regimen and M-G regimen

	A/S (N = 21)		M-G (N = 21)	
	n	%	*n*	%
Clinical cure	16	76	16	76
Clinical cure plus doxycycline	3	14	2	9
Failure (secondary to drug reaction)	1	5	0	0
Clinical failure	1	5	3	14

When the first patient had not responded to therapy within 3 days, a sonographic examination showed a cystic mass in the right adnexa. Clindamycin was added to the treatment regimen. The condition of the patient, however, did not improve and she underwent a unilateral salpingectomy. The second patient had bilateral tuboovarian masses and improved only after receiving clindamycin. The third patient had postabortal endomyometritis and was given cefoxitin after she had not responded to the M-G regimen within 48 hours. The infection was cured; however, analysis of laboratory results showed no reason why she had not responded to the initial medication.

Adverse reactions. A/S appeared to be better tolerated than M-G in this patient population. There were no adverse hematologic, renal, or hepatic side effects noted in either group. Four (18%) of the patients in the A/S group, compared with one (4.5%) of the patients in the M-G group, experienced some pruritus. However, eight (36%) patients in the M-G group experienced nausea and/or vomiting, compared with only two (9%) patients in the A/S group.

Microbiologic isolates. Eradication of the organisms isolated before treatment was better with the A/S combination than with M-G (Table V). The A/S regimen appeared to eradicate almost all of the organisms cultured. After treatment, only nine positive primary plate cultures were obtained from the endometrial cavity, as compared with 153 positive pretreatment cultures. However, after treatment with M-G, 55 positive pri-

Table V. A/S versus M-G in eradicating organisms isolated from study patients

Category of organism	A/S (N = 22)				M-G (N = 21)			
	Before treatment (N = 22)		After treatment (N = 21)		Before treatment (N = 21)		After treatment (N = 19)	
	No. of isolates from study patients	No. of isolates from primary plates	No. of isolates from study patients	No. of isolates from primary plates	No. of isolates from study patients	No. of isolates from primary plates	No. of isolates from study patients	No. of isolates from primary plates
Aerobic								
Gram-positive cocci	30	18	3	3	21	9	21	7
Gram-negative cocci	8	8	0	0	9	9	0	0
Gram-positive rods	22	17	4	2	19	14	30	23
Gram-negative rods	26	25	5	2	30	26	12	11
Anaerobic								
Gram-positive cocci	52	31	1	1	56	28	8	5
Gram-negative cocci	6	3	0	0	10	4	1	1
Gram-positive rods	13	8	3	1	13	6	7	5
Gram-negative rods	74	43	1	0	58	36	8	3
Total	231	153	17	9	216	132	87	55

Table VI. Minimum inhibitory concentrations of bacteria isolated from pretreatment cultures

Organism	Ampicillin		A/S		Metronidazole	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Black-pigmented *Bacteroides* sp. (n = 10*)	0.06	128.0	0.125	2.0	2.0	2.0
B. bivius (n = 17)	16.0	64.0	2.0	4.0	4.0	8.0
B. capillosus (n = 6)	8.0	8.0	1.0	1.0	2.0	4.0
B. disiens (n = 12)	0.5	8.0	0.25	1.0	4.0	8.0
B. fragilis group (n = 3)	16.0	16.0	1.0	2.0	1.0	2.0
Bacteroides sp. (n = 32)	0.5	32.0	0.25	1.0	2.0	8.0
Fusobacterium sp. (n = 8)	0.06	0.06	0.06	0.06	0.06	0.25
Mobiluncus (n = 4)	0.06	0.06	0.06	0.06	2.0	4.0

MIC_{50} = 50% minimum inhibitory concentration; MIC_{90} = 90% minimum inhibitory concentration.
*Number of isolates.

mary plate cultures were obtained from the endometrial cavity, compared with 132 before treatment. The organisms most frequently cultured after treatment were gram-positive and gram-negative aerobes. The failure to eradicate the organisms from primary plate cultures did not correlate with clinical failure or clinical response.

Minimum inhibitory concentration determinations were evaluated on bacteria isolated from pretreatment cultures. Ampicillin alone would have been ineffective against the major organisms responsible for the soft tissue pelvic infections in these patients, particularly *Bacteroides*. The addition of sulbactam lowered the minimum inhibitory concentrations and the A/S combination appeared to be more effective in vitro than metronidazole alone (Table VI). For example, the A/S combination reduced the minimum inhibitory concentration against black-pigmented *Bacteroides* from 128.0 with ampicillin alone to 2.0 with A/S. Similarly, the minimum inhibitory concentration against *B. bivius* was reduced from 64 to 4.0. The minimum inhibitory concentrations of metronidazole against black-pigmented *Bacteroides* and *B. bivius*, respectively, were 2.0 and 8.0. No significant enhancement of ampicillin by sulbactam against other non-*Bacteroides* organisms was seen.

Table VII. β-Lactamase production among *Bacteroides* isolates recovered from study patients

	β-Lactamase –positive	β-Lactamase –negative
Black-pigmented *Bacteroides*	5	5
B. asaccharolyticus	2	3
B. corporis	0	1
B. intermedius	1	1
B. levii	2	0
B. fragilis group	2	1
B. fragilis	1	1
B. vulgatus	1	0
B. bivius	16	1
B. capillosus	4	2
B. disiens	8	3
Bacteroides sp.	20	16
Total	55	28

Comment

Study results demonstrated that A/S is as effective as M-G in treating mixed aerobic-anaerobic pelvic infections. Moreover, the A/S combination appeared to be better tolerated than the M-G combination. It is important to recognize that metronidazole-aminoglycoside regimens will not be effective against some of the major pathogens responsible for pelvic infections, including chlamydia, group B streptococci, and other facultative streptococci. In addition, our study found that two thirds of the *Bacteroides* found in female genital tract infections were β-lactamase producers, against which ampicillin alone is ineffective.

Table VII shows β-lactamase production among the *Bacteroides* isolates recovered from our patients. Fifty-five (66%) of the 83 *Bacteroides* isolates cultured from the upper genital tracts of patients were β-lactamase producers. Five (50%) of the black-pigmented *Bacteroides* isolates were β-lactamase producers. Strikingly, 16 of 17 (94%) *B. bivius* organisms isolated from pelvic infections were β-lactamase producers. Similarly, 70% of the *B. capillosus* and *B. disiens* isolates were β-lactamase producers. More than half of the *Bacteroides* sp. thought to be sensitive to penicillin and first-generation cephalosporins were in fact β-lactamase producers. Without the use of the enzyme blocker sulbactam, ampicillin alone would have been ineffective in treating two thirds of the infections seen in these patients. Clearly, results of this study point out the need to include antimicrobial agents that are either resistant to β-lactamase or contain irreversible enzyme blockers against β-lactamase production.

The recovery of *Mobiluncus* sp. from the upper genital tract of patients with pelvic inflammatory disease is intriguing. *Mobiluncus* sp. have been implicated in bacterial vaginosis (formerly nonspecific vaginitis) as a component of the complex synergistic milieu of microorganisms involved in this entity, including *G. vaginalis,* anaerobic bacteria, α-hemolytic streptococci, and *M. hominis.*[15] Eschenbach[16] postulated that bacterial vaginosis may well be an antecedent precursor of acute pelvic inflammatory disease, especially the nongonococcal, nonchlamydial type. This hypothesis requires further investigation.

REFERENCES

1. Ledger WJ. Selection of antimicrobial agents for treatment of infections of the female genital tract. Rev Infect Dis 1983;5(suppl):S98.
2. Sweet RL. Treatment of mixed aerobic-anaerobic infections of the female genital tract. J Antimicrob Chemother 1981;8(suppl D):105.
3. Eschenbach DA, Buchanan TM, Pollack HM, et al. Polymicrobial etiology of acute pelvic inflammatory disease. New Engl J Med 1975;293:166.
4. Sweet RL. Anaerobic infections of the female genital tract. Am J Obstet Gynecol 1975;122:891.
5. Snydman DR, Tally FP, Knuppel R, Landrigan J, Grobach SL, Barrett JG. *Bacteroides bivius* and *Bacteroides disiens* in obstetrical patients: clinical findings and antimicrobial susceptibilities. J Antimicrob Chemother 1980;6:519.
6. Kirby BD, George WL, Sutter VL, Citron DM, Finegold SM. Gram-negative anaerobic bacilli: their role in infection and patterns of susceptibility to antimicrobial agents. I. Little known *Bacteroides* species. Rev Infect Dis 1980;2:914.
7. Joiner K, Lowe B, Dzink J, Bartlett JG. Comparative efficacy of ten antimicrobial agents in the experimental *Bacteroides fragilis* infections. J Infect Dis 1982;145:561.
8. Gall SA, Kohan AP, Ayers AM, Hughes CE, Addison WA, Hill GB. Intravenous metronidazole or clindamycin with tobramycin for therapy of pelvic infections. Obstet Gynecol 1981;57:51.
9. Gibbs RS, Blanco JD, Lipscomb KA, St Clair PJ. Aztreonam versus gentamicin, each with clindamycin, in the treatment of endometritis. Obstet Gynecol 1985;65:825.
10. Sweet RL, Ledger WJ. Cefoxitin: single agent treatment of mixed aerobic-anaerobic pelvic infections. Obstet Gynecol 1979;54:193.
11. Sweet RL, Robbie MO, Ohm-Smith M, Hadley WK. Comparative study of piperacillin versus cefoxitin in the treatment of obstetric and gynecologic infections. Am J Obstet Gynecol 1983;145:342.
12. Strausbaugh LJ, Liorens AS. Cefoperazone therapy for obstetric and gynecologic infections. Rev Infect Dis 1983;5:S154.
13. Wise R, Andrews JM, Bedford KA. Clavulanic acid and CP-45, 899: a comparison of their in vitro activity in combination with penicillins. J Antimicrob Chemother 1980;6:197.
14. Sweet RL, Ohm-Smith M, Landers DV, Robbie MO. Moxalactam versus clindamycin plus tobramycin in the treatment of obstetric and gynecologic infections. Am J Obstet Gynecol 1985;152:808.
15. Holmes KK, Spiegel C, Amsel R, et al. Nonspecific vaginosis. Scand J Infect Dis 1981;26(suppl):110.
16. Eschenbach DA. Epidemiology and diagnosis of acute pelvic inflammatory disease. Obstet Gynecol 1980;55(suppl): 142.

Infectious morbidity in gynecologic cancer

Doris C. Brooker, M.D., John E. Savage, M.D., Leo B. Twiggs, M.D., Leon L. Adcock, M.D., Konald A. Prem, M.D., and Christine C. Sanders, Ph.D.

Minneapolis, Minnesota, and Omaha, Nebraska

A retrospective investigation of infectious morbidity in gynecologic oncology patients documented that 54 (11%) of 494 patients and 68 (6%) of 1204 patient admissions were complicated by a serious infection. The highest rate of infectious morbidity by admission was 21%, occurring in patients admitted for cancer of the vulva. The highest surgical infectious morbidity, 22%, occurred in patients admitted for cervical cancer. Important factors in determining infection risk include multiple host factors, radical surgical procedures, factors inherent in the tumor itself, and additional irradiation and chemotherapy. These serious polymicrobial infections dictate intelligent selection of antimicrobials and appropriate monitoring to anticipate complications inherent in antimicrobial therapy. β-Lactamase induction, superinfection, nephrotoxicity, and necrotizing enterocolitis are documented problems in these patients. (Am J Obstet Gynecol 1987;156:513-20.)

Key words: Infectious morbidity, gynecologic cancer, genital tract malignancy

Malignancy of the female genital tract is an important and costly determinant in women's health care today. In 1986, cancer of the breast and lung will account for an estimated 18% and 19%, respectively, of cancer deaths in women.[1] Deaths from cancer of the uterus and ovary will total 4% and 5%, respectively. The incidence of cancer differs from the above mortality statistics. Breast malignancy accounts for 26% of all female cancer; cancers of the lung and uterus each have an incidence of 11%, and cancer of the ovary, 4%.

These figures reflect in part the stage at which malignancies are first recognized. Cancers of the cervix and uterus are diagnosed earlier, at a local stage of disease 44% and 78% of the time, respectively. In contrast, 49% of breast and 67% of lung malignancies are diagnosed after regional and distant spread has occurred in the patient. With the epidemiologic tools of the Papanicolaou smear, endometrial biopsy, and aggressive management of uterine bleeding and enlargement, the uterine cancer death rate has seen a steady decline since 1940. Deaths from breast and ovarian malignancy have remained fairly constant, but deaths from lung cancer have been rising at a logarithmic rate since 1970.

Infectious morbidity-mortality in gynecologic oncology has not been the subject of extensive investigation. An understanding of the basis of these infections requires an understanding of the multiple infection risk factors in the host, those in the extended radical surgical procedures, and factors inherent in the tumor itself.

Host factors include: (1) normal vaginal flora; (2) altered flora and organism virulence in malignancy; (3) altered flora from previous use of antimicrobials including induction of antimicrobial resistance and superinfection[2-7]; (4) general host defense factors such as mucosal barriers, cellular and humoral immunity, and normal and decreased neutrophil, lymphocyte, and macrophage function; (5) known surgical risk factors in women with malignancy such as older age, poor nutritional status (weight loss, obesity), and concurrent disease such as diabetes, hypertension, and bacterial or viral sexually transmitted disease; (6) previous host chemotherapy and/or radiation therapy.[8]

Risks derived from the radical surgical procedure include: (1) prolonged time of operation with the increased dissection and tissue damage of the extended "radical" procedure and presence of blood and exudate in the operative site and (2) anesthesia risk.[9-11]

The major risks arising from the tumor itself are: (1) preexisting subclinical infections in proliferating and necrotic malignancy and (2) possible immunosuppressive effects exerted by the tumor itself.[12-22]

In addition to the usual risks deriving from host factors, from the surgical procedures, and from the tumor itself, invasive diagnostic techniques used before operation add to the risk from the possibility of preexisting occult infection of the genital tissue and possible introduction of nosocomial infection. Invasive procedures such as cystoscopy, proctoscopy, needle biopsy, and dilatation and curettage are routine as are ma-

From the Division of Gynecologic Oncology and the Division of Gynecologic Pathology, Department of Obstetrics and Gynecology, University of Minnesota Medical School, and the Department of Medical Microbiology, Creighton University School of Medicine.

Supported by grants from Schering Corporation and Roerig, a division of Pfizer Pharmaceuticals.

Reprint requests: Doris C. Brooker, M.D., Box 395 UMHC, Department of Obstetrics and Gynecology, University of Minnesota Medical School, Minneapolis, MN 55455.

Table I. Complications of antimicrobial therapy

1. Organism selection, superinfection
2. β-Lactamase induction, organism resistance
3. Nephrotoxicity
4. Necrotizing enterocolitis from *Clostridium difficile*

Table II. Infectious morbidity

	No.	*%*
No. of patients with serious infection	54	11
Total No. of patients	494	
Admissions for infection	68	6
Total No. of admissions	1204	

Table III. Infectious morbidity by cancer site

Cancer site	*No. of infections per No. of admissions*	*Infectious morbidity (%)*
Cervix	26/325	8
Uterus	18/268	7
Ovary	12/436	3
Vulva	10/48	21
Other female genital malignancy	2/61	3

nipulative radiographic procedures such as intravenous pyelography, upper gastrointestinal series, barium enema, computerized axial tomographic scan, and ultrasound.

Finally, modern supportive procedures that add to postoperative infection risk include ureteral catheterization, percutaneous nephrostomy, central venous catheterization, hyperalimentation, and use of Jackson-Pratt drainage tubes, as well as urethral catheterization, insertion of a nasogastric tube, and placement of an intravenous line, all of which are common in patients after operation.[13, 14]

Given the lack of statistics in infectious morbidity and mortality in gynecologic malignancy, a retrospective descriptive investigation of the University of Minnesota Gynecologic Oncology Service was conducted for the year 1982 (January 1 through December 31, inclusive). Each admission was thoroughly examined for evidence of infection as well as the level of significance of infection. The infection was then correlated with the individual patient's malignancy, irradiation therapy, chemotherapy, surgery, and various other factors. The overall objective of the investigation was not only to compile a statistical file of infectious morbidity and mortality but also to use this file to develop specific and useful guidelines for prevention of infectious risk and

Table IV. Surgical infectious morbidity

Cancer site	*Total No.*	*No. infected*	*% Infected*
Cervix	73	16	22
Uterus	58	7	12
Ovary	55	6	11
Vulva	42	5	12
Vagina	10	1	10
Fallopian tube	1	1	–
Overall total	239	36	15

Table V. Host factor analysis: Age

Cancer site	*No. of patients*	*No. of admissions*	*Age (yr)*	
			Median	*Range*
Cervix	19	26	50	28-80
Uterus	15	18	65	47-90
Ovary	10	12	60	31-74
Vulva	8	10	75	53-88
Vagina	1	1	67	—
Fallopian tube	1	1	45	—
Total	54	68	—	—

for intelligent selection of antimicrobials. Ultimately these guidelines are to be put to use in prospective evaluations that lead to improved patient management.

The following discussion is a limited presentation of the 1982 investigation and is divided into three sections: first, general oncology data; second, infectious morbidity and mortality by cancer type, including general infection statistics, specific host factor analysis, and microbiology as well as serious infection by therapy mode; and third, the complications of antimicrobial therapy (Table I).

General oncology data

John L. McElvey Oncology Registry. The John L. McElvey Oncology Registry was created in the early 1940s and has maintained women's cancer statistics at the University of Minnesota without interruption since that time. In 1982, the Registry recorded 259 newly diagnosed malignancies, including 95 of the cervix, 73 of the uterus, 26 of the vulva, and 55 of the ovary. There were five new cases each of primary vaginal and gestational trophoblast disease.

Admissions by cancer site and therapy mode. There were a total of 1204 admissions to the service, including 436 (36%) for ovarian, 325 (27%) for cervical, 268 (22%) for uterine, 48 (4%) for vulvar, and 5% for other female genital malignancy. In addition, 66 (5%) admissions were for conditions other than malignancy. Of these 1204 admissions, 454 (38%) were for chemotherapy, 331 (27%) for operation, 173 (15%) for radiation therapy, and 93 (8%) for combinations of any of the above indications. Twelve percent of the admis-

Table VI. Host factor analysis: Length of stay (days)

Cancer site	Length of stay (days)		
	Average	Longest	Shortest
Cervix	17	43	3
Uterus	22	70	4
Ovary	12	37	2
Vulva	61	246	7
Vagina	59	—	—
Fallopian tube	23	—	—

Table VII. Host factor analysis: FIGO Stage for cervical cancer

Malignancy	Stage	No. of patients
Squamous cell carcinoma	IA	3
	IB	4
	II	2
	IIIB	2
	IV	2
Adenocarcinoma	IIB	1
	IV	1
Adenosquamous carcinoma	IB	1
	IIB	1
	IV	1
Polymorphic B-cell lymphoma (Epstein-Barr virus positive)	IV	1

sions involved evaluation only, with no therapeutic procedure.

Infectious morbidity and mortality

Serious infections by cancer type. A total of 494 individual patients accounted for the 1204 admissions. Fifty-four patients (11%) had a serious infection diagnosed during an admission. Ten patients had multiple admissions for the same infection (68 admissions for the 54 patients). Thus 6% (68 of 1204) of total admissions were complicated by serious infection (Table II). Uncomplicated urinary tract infections are not included in this tally.

There were 325 admissions for cancer of the cervix, and 26 (8%) of these were complicated by an infection. There were 268 admissions for uterine cancer, and 18 (7%) of these involved an infection. Three percent (12 of 436) of admissions for ovarian cancer were complicated by infection. Infectious morbidity for vulvar cancer was 21% (10 of 48) and for other female genital malignancy, 3% (two of 61) (Table III).

There were a total of 239 surgical procedures performed in 1982: 73 surgical procedures for cervical cancer (including 26 staging laparotomies, 21 radical hysterectomies, and two pelvic exenterations) and 58 surgical procedures for uterine malignancy (including 46 abdominal hysterectomies and one pelvic exenteration). Twenty-two percent (16 of 73) of cervical cancer patients had an infection complicating the admission for operation. Similarly, 12% (seven of 58) of uterine cancer patients, 11% (six of 55) of ovarian cancer patients, 12% (five of 42) of vulvar cancer patients, and 18% (two of 11) of patients with other gynecologic malignancy had an infection complicating the admission for operation (Table IV).

Host factor analysis. Selected characteristics of this patient population that contributed to the risk of serious infection included patient age, length of stay, and stage of disease (by International Federation of Gynecology and Obstetrics). The larger number of patients and admissions were for cervical and uterine cancer. As expected, vulvar, uterine, and ovarian cancer occurred more frequently in the older population (Table V).

The average length of stay (Table VI) for vulvar can-

Table VIII. Host factor analysis: FIGO Stage for uterine cancer

Malignancy	Stage	No. of patients
Adenocarcinoma	IA	2
	IB	4
	II	1
	III	1
	IV	2
Adenoacanthoma	IB	1
	III	1
Squamous cell carcinoma	II	1
Malignant mixed mesodermal tumor	III	1
Leiomyosarcoma, recurrent	IV	1

Table IX. Host factor analysis: FIGO Stage for ovarian cancer

	Stage	No. of patients
Nonmalignant (bilateral serous cystadenoma)	—	1
Malignant		
Serous cystadenocarcinoma	IIB	1
	III*	3
Adenocarcinoma	III*	2
Endometrioid adenocarcinoma	I	1
	II	1
Sex cord tumor, granulosa cell type	IV	1

*Suboptimal.

Table X. Host factor analysis: FIGO Stage for vulvar cancer

Malignancy	Stage	No. of patients
Squamous cell carcinoma	III	2
	IV	6

Table XI. Host factor analysis: Mortality

Cancer site	*No. of patients*	*FIGO Stage*	*Operation*	*Explanation*
Cervix	1	IV	Yes	Polymorphic B-cell lymphoma Renal transplant (1976) Peritonitis, mesenteric artery thrombosis
Uterus	2	IV	No	Pneumonia, gram-negative septicemia, myelosuppression
Ovary	3	III (1) IV (2)	Yes (2)	Septicemia, peritonitis
Vulva	1	IV	No	Myocardial infarction, gram-negative septicemia
Fallopian tube	1	III	Yes	Cardiomyopathy (4+) (Adriamycin toxicity), congestive failure, *Staphylococcus aureus* sepsis after Swan-Ganz, Hickman catheter placement

Table XII. Infections and organisms in cervical malignancy

Infection	*Organism*
Wound	*Staphylococcus epidermidis* Enterococci
Peritonitis	*Escherichia coli* *Klebsiella pneumoniae*
Pelvic hematoma and cellulitis	*Clostridium* sp. *Proteus mirabilis*
Subphrenic abscess	*Pseudomonas stutzeri* *Peptococcus asaccharolyticus*
Groin abscess	*Enterobacter aerogenes* *Enterobacter cloacae*
Jackson-Pratt drainage tube and wound site	*Candida albicans* *Peptococcus magnus* *Citrobacter freundii* *Staphylococcus aureus* Streptococci, group B *Haemophilus influenzae* *Bacteroides fragilis*
Urinary tract infection	*K. pneumoniae* *E. coli* Enterococci

cer patients far exceeded that of any other malignancy and accounted for the longest hospital stay. In the FIGO staging analysis (Tables VII to X), one of the most significant risk factors for infection was advanced stage of malignancy. More than 50% of cervical and uterine cancer patients had FIGO Stage II, III, or IV disease. The risk was even more pronounced in ovarian and vulvar cancer admissions, in which 85% had FIGO Stage III or IV disease.

Host factor analysis of mortality data (Table XI) showed that the eight patients who died during an admission all had FIGO Stage III or IV disease. Four of the eight died during an admission in which an operation-related infection occurred. However, all of the patients had other, more important complicating factors (myelosuppression, cardiomyopathy, advanced malignancy) that contributed to death.

Microbiology. The types of surgical infection found in gynecologic malignancy are shown in Tables XII to XV. These infections were typically polymicrobial, including both facultative gram-positive and gram-negative organisms as well as abundant anaerobes. The most likely source of these organisms was the altered vaginal flora.

Organisms such as *Pseudomonas, Enterobacter,* enterococci, *Candida, Clostridia,* and *Bacteroides* were frequently isolated in cases of significant infection (septicemia, pelvic cellulitis and hematoma, peritonitis, pneumonia, and pelvic abscess). In treating infections in which organism identification is not yet known, one must use empiric therapy that will cover this serious spectrum of organisms and anticipate the possible complications that may arise from such therapy.

During 1982 and 1983, in an investigation blinded to the evaluator, 13 gynecologic oncology patients with serious postoperative infections were prospectively randomized to netilmicin-clindamycin (N-C) or cefotaxime (C) therapy. All six N-C–treated patients responded promptly to treatment, while five of seven C-treated patients did not improve ($p = 0.02$). The investigation was terminated because single-agent therapy did not appear to treat these serious gynecologic infections effectively, especially in abscesses and deep tissue infections.

Infection risk by therapy mode. The risk of infection from operation and irradiation therapy was greatest in cervical (23 of 25) and uterine (21 of 30) cancer (Tables XVI and XVII). Chemotherapy was used more frequently in ovarian cancer patients than in cervical and uterine cancer patients. Chemotherapy and operation were almost equal risks for infection in ovarian cancer patients (Table XVIII). For vulvar cancer patients, operation posed the greatest risk (Table XIX). Recrudescence of *Herpesvirus,* a known risk in irradiation and chemotherapy, occurred in two of the 54 infected patients.

Table XIII. Infections and organisms in uterine malignancy

Infection	*Organism*
Necrotizing enterocolitis	*C. difficile*
Wound and skin infections	*Pseudomonas aeruginosa*
	S. epidermidis
	Enterococci
	Herpes zoster
	K. pneumoniae
	Veillonella parvula
Septicemia	*Clostridium sordellii*
	Enterococci
	S. epidermidis
	K. pneumoniae
	E. coli
Pelvic abscess	*K. pneumoniae*
	E. coli
Abdominal abscess	Enterococci
	Clostridium sp.
	Candida glabrata
	S. epidermidis
Pneumonia	*E. aerogenes*
	P. mirabilis
	K. pneumoniae
	C. albicans
Urinary tract infection	Enterococci
	P. aeruginosa
Pyelonephritis	*K. pneumoniae*
	E. coli
	P. mirabilis

Table XIV. Infections and organisms in ovarian malignancy

Infection	*Organism*
Septicemia	*S. epidermidis*
Wound	*S. aureus*
	Enterococci
Peritonitis	*Morganella morganii*
	E. coli
Pneumonia	*Aeromonas hydrophila*
	K. pneumoniae
	B. fragilis
	Clostridium perfringens
	Peptostreptococcus productus
	Veillonella parvula
	C. albicans
	P. aeruginosa
Urinary tract infection	*E. coli*
	C. albicans
Viral, secondary to chemotherapy	*Herpesvirus hominis*

Table XV. Infections and organisms in vulvar malignancy

Infection	*Organism*
Septicemia	*E. coli*
Inguinal and perineal wound	*S. aureus*
	S. epidermidis
	Stretococci, viridans group
Colostomy site	*E. coli*
	E. aerogenes
	P. aeruginosa
	B. fragilis
Urinary tract infection	*E. coli*
	Enterococci
	P. aeruginosa
	K. pneumoniae
	Klebsiella oxytoca

Complications of antimicrobial therapy

Several selected cases that highlight complications of antimicrobial therapy in gynecologic cancer patients are presented here. Complications discussed include: (1) antimicrobial resistance (β-lactamase induction), (2) organism selection (superinfection), (3) nephrotoxicity, and (4) necrotizing enterocolitis.

Case presentations

Case No. 1: β-Lactamase induction and antimicrobial resistance. The patient, a 74-year-old diabetic woman, had undergone radical vulvectomy and bilateral inguinal lymph node dissection. After operation, she developed a wound infection and urinary tract infection. She shared a whirlpool bath with patients from a nearby unit caring for burn patients. She was given 11 days of third-generation cephalosporin therapy.

Among other organisms, a type-specific *P. aeruginosa* was present in both groin wound infections and in the urinary tract infection. Initially sensitive to the cephalosporin, organisms became multiply resistant while the patient was receiving therapy. The β-lactamase inactivated the semisynthetic penicillins as well as multiple third-generation cephalosporins (Table XX). The inducible β-lactamase became constitutively produced (Table XXI). The emergence of multiple β-lactam resistance of the *Pseudomonas* in this patient was due to the in vivo selection of a mutant that was derepressed for β-lactamase production. Resistance mediated by this β-lactamase is via hydrolysis of substrate drugs and the nonhydrolytic barrier mechanism for poor or nonsubstrate drugs.[23, 24] Superinfection with yeast and enterococci also occurred during therapy.

Case No. 2: Organism selection and superinfection. The patient, an 83-year-old woman, had recurrent retroperitoneal low-grade leiomyosarcoma (primary in 1964) as well as pulmonary and cardiac disease. She underwent exploratory laparotomy and debulking of malignancy. She became febrile (temperature of 102.4° F) after 8 days of second-generation cephalosporin therapy.

Subclavian catheter tip and blood cultures taken at the febrile episode grew *S. epidermidis* and *E. aerogenes*. After treatment with tobramycin and vancomycin she became afebrile. A diagnosis of endocarditis was considered because cardiac and neurological compromise was evident, but the previous history of cardiac and pulmonary disease made definitive diagnosis difficult. Tobramycin and vancomycin were discontinued. She received another 8 days of second-generation cepha-

Table XVI. Serious infection risk factors in cervical cancer

Infection	*Non–cancer-related*	*Operation*	*Irradiation*	*Chemotherapy*
Fever of unknown origin	—	3	1	1
Abdominal wound	—	3	5 (1 patient)	—
Thoracic wound	—	1	—	—
Peritonitis	—	2	—	—
Pelvic hematoma, cellulitis	—	1	—	—
Subphrenic abscess	—	1	—	—
Groin abscess	1 (Obesity, diabetes)	—	—	—
Jackson-Pratt drainage, wound site	—	1	—	—
Urinary tract infection	—	1	3	—
Pneumonia	—	1	—	—
Subtotal	1	14	9	1

Table XVII. Serious infection risk factors in uterine cancer

Infection	*Terminal care*	*Operation*	*Irradiation*	*Chemotherapy*
Fever of unknown origin	—	1	3	—
Septicemia (blood culture positive)	1	1	2	—
Pelvic abscess, peritonitis	—	1	2	—
Abdominal wall tumor abscess and fistula	—	1	1	—
Pneumonia	1	1	—	—
Urinary tract infection	3	2	1	1
Pyelonephritis	1	—	1	—
Interstitial nephritis	—	1	—	—
Necrotizing enterocolitis	—	1	—	—
Wound/skin infection				
Back	—	—	—	1
Jackson-Pratt drain site	—	—	1	—
Perineal ulcers	—	—	1	—
Inguinal thigh (right) and buttocks (herpes zoster)	—	—	—	1
Subtotal	6	9	12	3

Table XVIII. Serious infection risk factors in ovarian cancer

Infection	*Operation*	*Chemotherapy*
Fever of unknown origin	3	—
Septicemia	—	2
Wound	1	—
Lymphangitis, groin (right) and vulva	1	—
Gluteal abscess (left)	—	1
Peritonitis	1	2
Pneumonia	—	1
Herpesvirus	—	1
Viral gastroenteritis	—	1
Urinary tract infection	1	1
Subtotal	7	9

Table XIX. Serious infection risk factors in vulvar cancer

Infection	*Operation*	*Irradiation*	*Chemotherapy*
Fever of unknown origin	1	—	—
Septicemia	—	1	—
Wound	7	—	—
Colostomy site	1	—	—
Perineal lymphangitis	—	—	1
Urinary tract infection	3	2	—
Subtotal	12	3	1

losporin therapy. She again became febrile with a temperature to 103° F. The patient had a urinary tract infection with *P. mirabilis*. Blood culture grew *S. epidermidis* and enterococci. The subclavian catheter tip grew *E. aerogenes, S. epidermidis,* and *P. mirabilis*. With ampicillin therapy, she became afebrile.

The antimicrobials were discontinued, and the patient again became febrile (temperature to 101.2° F). Blood culture grew enterococci; the subclavian catheter tip grew enterococci and *E. aerogenes*. At this time, there was evidence of valvular thickening, and the diagnosis of endocarditis was confirmed. The patient was begun on a regimen of long-term penicillin-gentamicin therapy.

Case No. 3: Nephrotoxicity. The patient, a 26-year-old woman, had squamous cell cancer of the cervix, FIGO Stage IV, diagnosed at the original operative pro-

Table XX. *P. aeruginosa* serotype 2: Results of broth dilution susceptibility tests*

Drug	*Minimum inhibitory concentration/minimum bactericidal concentration (μg/ml)*	
	PS1 (Before therapy)	*PS10 (Day 11)*
Carbenicillin	32/64	64/128
Azlocillin	4/64	64/128
Mezlocillin	8/32	64/128
Piperacillin	4/8	64/64
Aztreonam	4/8	16/32
Cefsulodin	1/8	8/64
Cefotaxime	16/16	>128/>128
Ceftazidime	1/16	8/8
Ceftriaxone	4/128	>128/>128
Moxalactam	8/32	64/128
N-Formidimidoyl thienamycin	4/4	1/8
Gentamicin	≤0.12/0.25	≤0.12/1
Tobramycin	≤0.12/≤0.12	≤0.12/0.25
Netilmicin	≤0.12/0.5	0.5/2.0
Amikacin	0.5/2.0	0.5/1.0

*Mueller-Hinton broth, 3 ml fixed volume, inoculum of 10^5 cfu/ml.

Table XXI. Results of β-lactamase assay (whole cell assay, ultraviolet spectrophotometric method)

Substrate	*β-Lactamase activity*			
	Before therapy (inducible)		*Day 11 of therapy (constitutive, derepressed)*	
	PS1	*PS2*	*PS10*	*PS11*
Cephalothin	17/99*	13/99	99/99	99/99
Cefamandole	0/8	0/8	0/10	0/12
Cefoxitin	0/0	0/0	0/0	0/0
Cefotaxime	0/0	0/0	0/0	0/0

*Nanomoles of substrate inactivated per 10^9 cells; values shown are for uninduced cells/cells after overnight induction by cefoxitin.

cedure. She underwent exploratory laparotomy and colostomy. Previously she had undergone abdominal and pelvic irradiation and cisplatin chemotherapy. She developed a subphrenic abscess after operation and was treated with aminoglycoside. During therapy, she developed documented aminoglycoside nephrotoxicity that was reversible when antimicrobial therapy was discontinued.

Case No. 4: Nephrotoxicity. The patient, a 61-year-old diabetic woman, had adenocarcinoma of the endometrium, FIGO Stage IB. Total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed with subsequent pelvic irradiation. She developed *K. pneumoniae* septicemia from an infected lymphocyst. One week after completion of aminoglycoside therapy, she developed reversible nephrotoxicity. Renal function returned to normal in 1 month.

Case No. 5: Necrotizing enterocolitis. The patient, a 77-year-old woman, had adenocarcinoma of the endometrium (FIGO, Stage IA), melanoma, and right hypernephroma. She underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, and right radical nephrectomy. Second-generation cephalosporin therapy was given for a total of 7 days after operation.

The first postoperative complication consisted of interstitial nephritis and renal failure induced by the cephalosporin and reversible on discontinuation of the antimicrobial. The second complication was diarrhea secondary to necrotizing enterocolitis. Toxicogenic *C. difficile* and its toxin were identified in the stool. The patient was given vancomycin, and the infection resolved. She then developed a urinary tract infection, from which *P. aeruginosa* was isolated. This infection resolved with semi-synthetic penicillin therapy.

Comment

Serious infections are a frequent occurrence in gynecologic oncology patients. Intelligent selection and appropriate monitoring of antimicrobial therapy are vital in preventing complications, which can include β-lactamase induction, superinfection, nephrotoxicity, and necrotizing enterocolitis.

We wish to thank Loretta Leman and Anne Shepard for consistent research data collection and evaluation throughout this project.

REFERENCES

1. Silverberg E, Lubera J. Cancer statistics, 1986. CA 1986; 36:9.
2. Ohm MJ, Galask RP. Bacterial flora of the cervix from two prehysterectomy patients. AM J OBSTET GYNECOL 1975;122:683.
3. Mead PB. Cervicovaginal flora of women with invasive cervical cancer. Obstet Gynecol 1978;52:601.
4. Ohm MJ, Scott JR, Galask RP. Cervical-vaginal flora of immunosuppressed renal transplant patients. AM J OBSTET GYNECOL 1978;130:49.
5. Gorbach SL, Medna KB, Thadepalli H, et al. Anaerobic microflora of the cervix in healthy women. AM J OBSTET GYNECOL 1973;117:1053.
6. Gilstrap LC, Gibbs RS, Michel TJ, et al. Genital aerobic bacterial flora of women receiving radiotherapy for gynecologic malignancy. Gynecol Oncol 1986;23:35.
7. Gerstner GJ, Kucera H. Weghaupt K, et al. Endometrial bacteria in patients with endometrial cancer before and after primary intracavitory irradiation using IR-192 and an afterloading technique. Arch Gynecol 1982;231:299.
8. Barber HRK. The critically ill patient with gynecologic cancer. Clin Obstet Gynecol 1985;28:414.
9. Marsden DE, Cavanagh D, Wisniewski BJ, et al. Factors affecting the incidence of infectious morbidity after radical hysterectomy. AM J OBSTET GYNECOL 1985;152:817.
10. Mann WJ, Orr JW, Shingleton HM, et al. Perioperative influences on infectious morbidity in radical hysterectomy. Gynecol Oncol 1981;11:207.
11. Rosenshein NB, Ruth JC, Villar J, et al. A prospective randomized study of doxycycline as a prophylactic antibiotic in patients undergoing radical hysterectomy. Gynecol Oncol 1983;15:201.
12. Larsen B, Lifshitz S, Galask RP. Infectious diseases in gynecologic malignancy. In: Buchsbaum HJ, Sciarra JJ, eds. Gynecologic oncology. New York: Harper & Row, 1981:1-11, 19.
13. Morgan LS, Daly JW, Monif GRG. Infectious morbidity associated with pelvic exenteration. Gynecol Oncol 1983;10:318.
14. Scanlon EF. Infection following radical pelvic surgery. Gynecol Oncol 1986;23:125.
15. Chambers JT, Kapp DS, Lawrence R, et al. Intermediate vs. delayed hysterectomy for endometrial carcinoma: surgical morbidity and hospital stay. Obstet Gynecol 1985;65:245.
16. Hancock KC, Copeland LJ, Gershenson DM, et al. Urinary conduits in gynecologic oncology. Obstet Gynecol 1986;67:680.
17. Daly JW, Pomerance AJ. Groin dissection with prevention of tissue loss and postoperative infection. Obstet Gynecol 1979;53:395.
18. Duff P, Gibbs RS. Pelvic vein thrombophlebitis: diagnostic dilemma and therapeutic challenge. Obstet Gynecol Surv 1983;38:365.
19. Fox LP. Fatal superinfection with monilia in gynecological surgery. AM J OBSTET GYNECOL 1971;110:285.
20. Sweet RL, Yonekura ML, Hill G, et al. Appropriate use of antibiotics in obstetrics and gynecologic infections. AM J OBSTET GYNECOL 1983;146:719.
21. Cartwright PS, Pittaway DE, Jones HW III, et al. The use of prophylactic antibiotics in obstetrics and gynecology: a review. Obstet Gynecol Surv 1984;39:537.
22. Sevin B, Ramos R, Lichtinger M, et al. Antibiotic prevention of infections complicating radical abdominal hysterectomy. Obstet Gynecol 1984;64:539.
23. Sanders CC, Novel resistance selected by the new expanded-spectrum cephalosporins: a concern. J Infect Dis 1983;147:585.
24. Medeiros AA. β-Lactamases. Br Med Bull 1984;40:18.

CORRESPONDENCE

Surveillance of twin pregnancy with umbilical arterial velocimetry

To the Editors:

We read with interest the paper by Farmakides et al. (AM J OBSTET GYNECOL 1985;153:789-92) in which they report their results of umbilical artery Doppler studies in twin pregnancies.

Farmakides et al. conclude that the twin fetus of average for gestational age has elevated umbilical vascular resistance compared to the singleton fetus. We have not found this to be the case in our study using the same technique nor did Giles et al.[1] in their study of twin pregnancy.

This discrepancy can be explained by the fact that Farmakides et al. categorized their fetuses as being of average or small for gestational age by means of inappropriate criteria. They used data from Naeye[2] which was collected solely from twins who were stillborn or died in the first week of life. As many as 30% of perinatal deaths in twins result from intrauterine growth retardation and asphyxia; thus the mean birth weight in Naeye's population is considerably less than is usually recorded for twins and therefore cannot be used to determine whether a twin fetus is average or small for gestational age.

Their average for gestational age population would include a number of fetuses that would be considered small for gestational age by singleton criteria, and this explains both the apparent low incidence of growth-retarded fetuses (10%) and the elevated systolic/diastolic ratio in their average for gestational age population.

J. W. K. Ritchie, M.D.
Lesley McCowan, M.B.

University of Toronto
Mount Sinai Hospital
600 University Avenue
Toronto, Ontario, Canada M5G 1X5

REFERENCES

1. Giles WB, Trudinger BJ, Cook CM. Fetal umbilical artery flow velocity time waveforms in twin pregnancies. Br J Obstet Gynaecol 1985;92:490.
2. Naeye RL. The fetal and neonatal development of twins. Pediatrics 1964;33:546.

Reply

To the Editors:

We appreciate the continuing interest of Ritchie and McCowan in our work. We also look forward to the publication of their data so that we can compare differences in results.

As indicated in their letter, since our results apparently differ from those of Giles et al., Ritchie and McCowan offer the suggestion that our choice of weight standards may explain the differences. They may be right, but as they are well aware, there is no general agreement about which standard to use for either a single or twin fetus when defining growth retardation.

There are many other reasons for differences in results. In addition to the choice of weight standards, one could add (1) statistical methods, (2) use of Doppler device, (3) use of spectrum analyzer, (4) choice of subjects, and (5) size of sample just to mention a few.

The road to truth is a tortuous one. This is a new area of investigation. Our study is a beginning. The essential point is that Doppler velocimetry will identify the twin fetus at risk for death or damage from inadequate umbilical flow. This is a valuable addition to our surveillance methodology. The exact numbers will be revealed from continuing study of more women.

George Farmakides, M.D.
Harold Schulman, M.D.

Winthrop-University Hospital
State University of New York at Stony Brook
Mineola, Long Island, New York 11501

Additional statistical analysis of antenatal phenobarbital for prevention of neonatal intracerebral hemorrhage

To the Editors:

We read with interest the report by Shankaran et al. (AM J OBSTET GYNECOL 1986;154:53-7) regarding the prevention of intraventricular hemorrhage with antenatal phenobarbital. We would like to question one of the statistical conclusions and then to offer some additional analysis.

In column 1 of page 55 they say, "Among infants who developed intracerebral hemorrhage, significantly more control infants were delivered vaginally." Examination of the data reveals the following: of the 13 control infants who had hemorrhage, seven were delivered vaginally and six nonvaginally; of the eight treated infants with hemorrhage, two were delivered vaginally and six nonvaginally (Fisher's exact test, $p = 0.201$). We wonder if the intended assertion was, "Of infants delivered vaginally, significantly more control infants developed hemorrhage." Examination of the data concerning that statement reveals the following: of 13 control infants delivered vaginally, seven had hemorrhage and six did not; of 14 treated infants delivered vaginally, two had hemorrhage and 12 did not ($p = 0.037$).

Detailed statistical analysis was not presented on mechanical ventilation as a confounding variable. Of 32 infants ventilated, 20 developed some form of hemorrhage; of 16 infants not ventilated, only one had some form of hemorrhage ($p = 0.0007$, χ^2). Of control infants who hemorrhaged, it appears that those who were mechanically ventilated may have had significantly more moderate/severe hemorrhage compared to those

who were not ventilated ($0.47 < p < 0.096$, Fisher's exact test; the precise value could not be determined from the data presented). The degree of mechanical ventilation and severity of respiratory distress are also impossible to factor from the data presented.

These issues aside, the fact still remains that there was a significant difference in the number of moderate/severe hemorrhages between those infants whose mothers received phenobarbital and those who did not. This difference was present despite serum phenobarbital levels well below the "anticonvulsant levels" used by Donn et al.[1] and Morgan et al.[2] Donn et al. found a decreased incidence of grade I and II hemorrhages in infants treated postnatally with phenobarbital. Morgan et al. found no differences in either mild or severe hemorrhage after postnatal administration of phenobarbital. We wonder whether the effect seen with antenatal phenobarbital administration is due to effects on labor patterns (frequency, force, and duration of contractions) and not directly on the infant. Future studies in which the labor history is more completely documented might help clarify this issue. Inclusion of data regarding the use of tocolytics and steroids would also be beneficial.

We applaud the authors for their work and certainly agree that further prospective randomized trials of antenatal phenobarbital should be carried out.

Robert V. Jarrett, D.O.
Glen D. Jordan, M.D.

Department of the Army
Madigan Army Medical Center
Tacoma, Washington 98431-5000

REFERENCES

1. Donn S, Roloff D, Goldstein G. Prevention of intraventricular haemorrhage in preterm infants by phenobarbitone. Lancet 1981;2:215.
2. Morgan M, Massey R, Cooke R. Does phenobarbitone prevent periventricular hemorrhage in very low-birth-weight babies?: a controlled trial. Pediatrics 1982;70:186.

Reply

To the Editors:

We thank Dr. Jarrett for his careful review of our paper and for correcting our error on column 1 of page 55. Of the 13 control infants who were delivered vaginally, seven developed hemorrhage while six did not, and among the 14 treated infants delivered vaginally, two developed hemorrhage and 12 did not ($p < 0.05$). Hence the intended assertion was "Of the infants delivered vaginally, significantly more control infants developed hemorrhage." The vaginal mode of delivery has been shown to be a risk factor for hemorrhage by other investigators.

We have done more analysis of the relation between mechanical ventilation and the degree of hemorrhage in the control and treated groups of infants (see Table I). When evaluating the infants who did not require mechanical ventilation, we found no significant difference in the incidence of hemorrhage versus no hemorrhage or no and mild hemorrhage versus moderate and severe hemorrhage between the control and the treated groups of infants. On the other hand, when we evaluated infants who required mechanical ventilation, there was a significant difference in the incidence of intraventricular hemorrhage between the treated and control infants when comparing no hemorrhage versus hemorrhage ($p = 0.02$) and no and mild hemorrhage versus moderate and severe hemorrhage ($p = 0.003$). Hence phenobarbital was not protective among the nonventilated group (who had a very low incidence of hemorrhage) but was protective against moderate and severe hemorrhage in the ventilated group. Infants requiring mechanical ventilation have been shown to be at a great risk for intraventricular hemorrhage.

We can only speculate on the mode of action of antenatal phenobarbital administered in the 500 mg dose. The levels achieved were lower than those used in all other studies evaluating the effects of postnatal phenobarbital therapy and were not associated with any complications. We are in the second phase of our study and are evaluating the effects of phenobarbital on labor patterns, as well as the effect of tocolytics and β-methasone on incidence of intraventricular hemorrhage. We thank Dr. Jarrett for his helpful suggestions.

Seetha Shankaran, M.D.
Eugene E. Cepeda, M.D.
Ronald L. Poland, M.D.

Hutzel Hospital and Children's Hospital of Michigan
Department of Pediatrics
Wayne State University School of Medicine
Detroit, Michigan 48201

Table I. Relation between mechanical ventilation and the degree of hemorrhage in the control and phenobarbital-treated groups of infants

	*Mechanical ventilation**		*No ventilation*	
Hemorrhage	*Treated infants*	*Control infants*	*Treated infants*	*Control infants*
None	10	2	7	8
Mild	8	6	0	1
Moderate/severe	0	6	0	0

*No hemorrhage vs. hemorrhage, $p < 0.05$. No and mild hemorrhage vs. moderate and severe hemorrhage, $p < 0.01$.

Intrapartum fetal heart rate monitoring

To the Editors:

In their randomized controlled trial of continuous electronic versus intermittent auscultatory fetal heart rate monitoring during labor (AM J OBSTET GYNECOL 1986;152:524-39), MacDonald and his colleagues from the National Perinatal Epidemiology Unit have suggested that continuous electronic monitoring is superior in reducing the incidence of seizures in the neonate. They deserve our congratulations and gratitude for undertaking so large a trial and "policing" it so well; and I hope I am not being merely curmudgeonly in pointing out what seems to me an important flaw in their opus magnum.

For a randomized controlled trial to be utterly convincing, it needs to be more than large and well conducted. It must also stimulate confidence that both groups of patients were treated with the same will and determination to achieve the best possible results for the patients in them. In the recent Dublin trial the very protocol for the management of the intermittent auscultation group ensures that maximal results for the patients in this group were impossible to achieve. Arguably, the best sign of fetal distress that can be detected by intermittent auscultation is slow recovery of the fetal heart rate after contractions and this entails auscultating the fetal heart rate by Pinard stethoscope (or Doppler ultrasound, if necessary) both during and after contractions. I was taught this during my first resident obstetric job over 20 years ago, and it remains routine practice with the midwives in the units with which I am currently associated. By insisting in their protocol that the fetal heart rate be auscultated "following a contraction" or during the "interval between contractions," the Dublin workers have ensured that this major sign of fetal distress will have been missed until severe and prolonged enough to present as a profound baseline bradycardia. This single and simple fact could account for the very low incidences of fetal heart rate abnormality requiring electronic monitoring (0.49%) or fetal blood sampling (0.6%) in the intermittent auscultation group, the longer duration of labor in this group, and the higher incidence of neonatal seizures.

I wonder if MacDonald et al. have considered this severe limitation to the practical value of their work?

D. D. Mathews, M.D.
All Saints' Hospital
Chatham, Kent, England ME4 5NG

Reply

To the Editors:

Dr. Mathews believes that the method of intermittent auscultation of the fetal heart which he uses is superior to the method evaluated in the Dublin randomized trial. We have also heard that other obstetricians believe that their methods of interpreting fetal heart rate traces are superior to those evaluated in the trial. These beliefs should be seen for what they are: clinical opinions that cannot (so far) be supported with evidence from properly controlled clinical experiments.

Dr. Mathews has already tested the validity of a number of his beliefs in other areas of obstetric practice by mounting randomized trials.[1-6] We hope that he and others who believe that more effective methods of intrapartum fetal monitoring exist than those compared in the Dublin trial will apply similar scientific self-discipline in respect of these beliefs.

Dermot MacDonald
Adrian Grant
Margaret Sheridan-Pereira
Peter Boylan
Iain Chalmers

National Perinatal Epidemiology Unit
Radcliffe Infirmary
Oxford, England OX2 6HE

REFERENCES

1. Mathews DD, Friend JB, Michael CA. A double-blind trial of oral isoxsuprine in the prevention of premature labour. J Obstet Gynaecol Br Cmmwlth 1967;74:68-70.
2. Mathews DD, Loeffler FE. The effect of abdominal decompression on fetal oxygenation during pregnancy and early labour. J Obstet Gynaecol Br Cmmwlth 1968;75: 268-70.
3. Mathews DD, Hossain H, Bhargava S, D'Souza F. A randomised controlled trial of an oral solution of prostaglandin E2 and oral oxytocin used immediately after low amniotomy for induction of labour in the presence of a favourable cervix. Curr Med Res Opin 1976;4:233-40.
4. Mathews DD. A randomized controlled trial of bed rest and sedation or normal activity and non-sedation in the management of non-albuminuric hypertension in late pregnancy. Br J Obstet Gynaecol 1977;84:108-14.
5. Mathews DD, Agarwal V, Shuttleworth TP. The effect of rest and ambulation on plasma urea and urate levels in pregnant women with proteinuric hypertension. Br J Obstet Gynaecol 1980;87:1095-8.
6. Mathews DD, Agarwal V, Shuttleworth TP. A randomized controlled trial of complete bed rest versus ambulation in the management of proteinuric hypertension during pregnancy. Br J Obstet Gynaecol 1982;89:128-31.

Transcervical chorionic villus sampling

To the Editors:

We feel obliged to respond to the article entitled "A randomized study of three cannulas for transcervical chorionic villus sampling" (MacKenzie et al. AM J OBSTET GYNECOL 1986;154:34-9) because it presents the Portex catheter as an ineffective sampling device. (Incidentally, in Fig. 1 the picture of the Portex Trophocan is incomplete and only shows the obturator—the catheter itself is missing).

The Portex catheter is the instrument used in most of the centers reporting to the World Health Organization Registry.[1] This shows that in nearly 12,000 diagnostic cases performed at 90 centers worldwide, there has been an overall rate of failure of diagnosis of 2.6% and a maximum fetal loss rate of 4%, falling to <2% at experienced centers. Most of the samples have been taken for karyotyping.

By contrast, Mackenzie et al. achieved only a 60% success rate in obtaining a sample of villous material and only a 16% success rate in obtaining a karyotype with use of the Portex catheter.

The discrepancy between the experiences of the authors and the rest of the world is probably due to differences in technique; for instance, it appears that they do not inspect the sample for the presence of an adequate amount of chorionic villi at the bedside. This inspection is necessary for the rapid feedback required to perfect the obstetrician's skill and to maintain a high rate of sampling success[2] and is a nearly universal feature of diagnostic sampling procedures.

A second objective of the study was to simplify the procedure by having one operator perform both the scanning and the sampling, but this issue has already been competently addressed by Rodeck et al.[3] in 1983.

We all recognize that there are problems associated with transcervical chorionic villus sampling, and the development of the transabdominal route is one that we support.[4]

It seems likely that the authors' difficulties could have been resolved in the conventional way by discussion with scientific colleagues. It is a pity to resort instead to designing yet more sampling devices and publishing a report that, to the reader who does not actually practice chorionic villus sampling, seriously misrepresents the current state of the art.

R. H. T. Ward
B. Modell
M. Petrou

Department of Obstetrics and Gynaecology
Faculty of Clinical Sciences
University College, London
88-96 Chenies Mews
London, England WC1E 6HX

REFERENCES

1. Jackson L, ed. Chorionic Villus Sampling Newsletter March 6, 1984:4.
2. Ward RHT, Modell B, Petrou M, Karagözlu F, Douratsos E. Method of sampling chorionic villi in first trimester of pregnancy under guidance of real time ultrasound. Br Med J 1983;286:1542.
3. Rodeck CH, Morsman JM, Nicholaides KH, MacKenzie C, Gosden CM, Gosden JR. A single-operation technique for first trimester chorionic biopsy. Lancet 1983;2:1340.
4. Ward RHT, Modell B, Petrou M. Transabdominal chorionic villus sampling. Lancet 1986;1:440.

Reply

To the Editors:

We are grateful for the interest shown by Ward et al. in our article.

At no point do we claim that it is impossible to obtain chorionic villi with a high degree of success with use of a Portex cannula in the diagnostic situation. It is always possible to obtain villi given the right set of circumstances with use of practically any cannula. The point of this study was to determine whether by applying a certain defined set of circumstances in a randomized manner to each of three different cannulas there was any detectable variation in the weight of villus and the number of successful karyotypes obtained.

The technique followed was exactly that recommended in the leaflet that accompanied the Portex Trophocan device. Before the study one of the authors had performed biopsies in a large series of patients with use of the Portex, so that experience with the technique was deemed to be adequate.

The main criticisms leveled at the authors seem to be (1) that the sample obtained was not inspected for an adequate amount of villi at the bedside and (2) that "difficulties" could have been resolved by discussion with scientific colleages. We would like to reply to these points.

First, each sample was inspected for the presence of an adequate amount of chorionic villi at the bedside. Such an inspection does not invalidate the results, however, since the number of passes made with each catheter had already been decided before each sampling and the presence or absence of villi could have no effect on this. In other words, the results were not permitted to bias the manner of the experimentation.

Second, when diagnostic samples are being undertaken the authors do "address their problems in the conventional way." The outcome of a visit to King's College Hospital was a modification in the suction procedure which we believe would have improved the yield of villi obtained.

Consequently in our diagnostic series of patients receiving biopsies by transcervical chorionic villus sampling with use of an aluminum cannula, the success rate in obtaining a karyotype by means of direct preparations of these samples is 100%.

Thus our point still stands that under direct comparable circumstances when the limiting factor is the type of cannula employed, the aluminum type proved to give the most satisfactory results.

W. E. Mackenzie
D. S. Holmes, Ph.D.
T. Webb, Ph.D.
J. R. Newton, M.D.

Birmingham Maternity Hospital
Queen Elizabeth Medical Centre
Edgbaston, Birmingham, England B15 2TG

MATERNAL-FETAL MEDICINE

Berkshire Medical Center
Pittsfield, Massachusetts

A rapidly growing Perinatal Program is recruiting a second full time faculty subspecialist in Maternal-Fetal Medicine effective July 1, 1987. Responsibilities include consultations and care of high-risk patients, genetic counseling and testing, level II ultrasound, resident and student teaching.

Berkshire Medical Center is a 365-bed Regional Referral Medical School and a major teaching center affiliated with the University of Massachusetts. The position provides an excellent opportunity to develop a state-of-the-art program in Maternal-Fetal Medicine in a suburban setting. Competitive salary arrangement.

If you are board eligible or certified in Maternal-Fetal Medicine, contact: Shaul Yarkoni, M.D., Chairman, Department of Obstetrics and Gynecology, (413) 499-4161, ext. 2202.

An equal opportunity employer

Berkshire Medical Center
BERKSHIRE HEALTH SYSTEMS
725 North Street, Pittsfield, MA 01201

Join the Group in Minneapolis.

Outstanding career opportunity combining superior care with professional growth. Join progressive department of 20 physicians, 12 nurse-midwives and 6 ob/gyn nurse practitioners delivering 300 babies each month and providing family planning, infertility, high-risk obstetrics, laser surgery, colposcopy, ultrasonography, laparoscopy and maternal/fetal medicine in one of America's leading metropolitan areas.

Comprehensive benefits including professional liability, four weeks vacation, two weeks conference leave, sick leave, medical coverage, dental coverage, life insurance, disability and retirement plans. Opportunities for teaching and university affiliation. Please send c.v. to: **M. Aksoy, M.D., Chairman, Department of Obstetrics and Gynecology; GROUP HEALTH, INC.; 2829 University Ave. S.E., Suite 220; Minneapolis, MN 55414.**

CHIEF of OBSTETRICS

Specialist in MATERNAL-FETAL MEDICINE

Immediate opening at HARVARD COMMUNITY HEALTH PLAN for senior Obstetrician to supervise the Brigham & Women's Hospital HCHP delivery service. Will serve on hospital's executive committee.

STAFF Obstetricians — several positions for our staff and group model HMO practices. Serving over 300,000 New England residents, we offer excellent benefits and salary commensurate with experience. Board Certified or Eligible candidates please contact Marc Bard, M.D., HCHP, One Fenway Plaza, Boston, MA 02215, 617-731-8275

AA/EO Employer

Harvard Community Health Plan

Maternal-Fetal Medicine

Board certified/eligible PERINATOLOGISTS required for large volume obstetric services (3,000-6,000 deliveries per year).

Opportunities exist both to join and create high risk obstetric services at various Kaiser Permanente Medical Care Centers located in Southern California. University affiliations and clinical research is encouraged.

Excellent benefits are offered including liability coverage, medical and dental coverage, vacation and sick leave, continuing education and retirement program. Partnership consideration available after two years full time employment.

Please write, including your curriculum vitae and three professional references to:

KAISER PERMANENTE
SOUTHERN CALIFORNIA
PERMANENTE MEDICAL GROUP

William G. McCormick
Physician Recruitment
Dept. 41B
Walnut Center
Pasadena, CA 91188
(714)829-5365
Equal Opportunity Employer M/F/H

OBSTETRICIAN GYNECOLOGIST-IN-CHIEF

The Mary Imogene Bassett Hospital, a 189-bed, rural referral center, and major teaching affiliate of Columbia University, is seeking candidates for this key appointment. The chief is responsible for overall clinical and teaching programs in Obstetrics and Gynecology. The Service is providing approximately 500 deliveries and about 4300 obstetrical clinic visits yearly. Volume in Gynecology is about 290 admissions and 7800 clinic visits. The department has four attending physicians, rotating transitional residents, a certified nurse midwife, a physician's assistant, plus substantial nursing and office staffing.

Cooperstown, on scenic Lake Otsego about 60 miles west of Albany, enjoys unusual recreational opportunities, a strong school system, and an array of cultural and artistic activities. The village is known for its air of history, museums and resort facilities.

The medical staff is organized as a salaried group practice with full fringe benefits including malpractice coverage, generous free time, conference leave, and immediate annuity participation.

Please send curriculum vitae and correspondence to:

Harlan D. Alpern, M.D.
Search Committee Chairman
The Mary Imogene Bassett Hospital
Cooperstown, New York 13326
telephone (607) 547-3700

THE MARY IMOGENE
BASSETT HOSPITAL

Faculty Position GEORGIA

Chief, OB/GYN in Community Hospital. Academic setting teaching Family Practice Residents who staff busy OB/GYN service in tertiary perinatal center. Emory University and Medical College of Georgia affiliate. Relaxed professional environment.

Send C.V. to:

Howard G. Vigrass, M.D.
710 Center Street
Box #84
Columbus, GA 31994

REPRODUCTIVE ENDOCRINOLOGY RESEARCH FELLOWSHIP

This research fellowship, beginning July 1987, is designed for gynecologists who are already board-eligible in the Reproductive Endocrinology and Infertility Subspecialty by the American Board of Obstetrics/Gynecology. This 2-3 year research fellowship will equip the candidate with specific training in the laboratory aspects of clinical and basic research in Reproductive Endocrinology. It is specifically aimed at those choosing academic careers who feel the need for further research training upon completion of their clinical training. For information, please write to George S. Richardson, M.D., Acting Chief, Dpt. of Gynecology, Vincent 1, Massachusetts General Hospital, Boston, MA 02114.

PACIFIC NORTHWEST

Practice opportunity available in Arlington, Washington in Obstetrics/Gynecology

New 48-bed hospital (to replace existing facility) under construction, will open summer 1987; office space available adjacent to hospital. Service area is approximately 45,000. Cascade Valley Hospital is affiliated with Virginia Mason Medical Center in Seattle.

Candidates must be board certified or intending to become certified within one year. Must also desire to live in the Arlington area.

Arlington is a rural community of 3,000 located 45 minutes north of Seattle at the foothills of the Cascades and 20 minutes from Puget Sound. The area offers excellent schools and outdoor recreational opportunities.

Please send CV to Patty House, Health Resource Services, 925 Seneca Street, Box 1930, Seattle, WA 98111. (206) 223-6351.

MRHP **Michael Reese Health Plan**

A LARGE HOSPITAL-BASED HMO

has openings for the following full-time position:

Obstetrician Gynecologist

THE MICHAEL REESE HEALTH PLAN, a well-established and rapidly-expanding, federally-qualified HMO seeks Board Certified/Eligible Obstetrician/Gynecologists.

THIS POSITION entails both outpatient and hospital practice as well as medical students and house staff teaching.

REMUNERATION is high and competitive with academic and fee-for-service practice markets. Fringe benefits are generous.

INTERESTED APPLICANTS should send Curriculum Vitae in confidence to:
Dr. Stephen Contro, Medical Director, MICHAEL REESE HEALTH PLAN, 2545 Martin Luther King Drive, Chicago 60616

an equal opportunity employer m/f/h

FACULTY POSITIONS MATERNAL FETAL-MEDICINE

The Department of Obstetrics and Gynecology at the Medical College of Ohio at Toledo, is accepting applications for full time faculty positions within the Division of Maternal Fetal Medicine. The Center for Women and Children is the level III Perinatal Referral Center for Northwest Ohio and has over 4400 deliveries yearly. There is a 60 bed NICU with 6 full time neonatologists. The candidate should be board certified or board eligible in Maternal Fetal Medicine. Duties will include patient care, teaching activities with residents and medical students and development of research activities. Salary and academic rank will be commensurate with the candidate's training and experience. Please direct inquiries and curriculum vitae to:

Roger R. Lenke, M.D.
Associate Professor
Director Maternal Fetal Medicine
Medical College of Ohio
C.S. 10008
Toledo, Ohio 43699
419-381-4591

PERINATOLOGIST
Denver, Colorado

Rose Medical Center, Denver, Colorado, is seeking a BE/BC perinatologist to work full-time in a 400-bed private community hospital adjacent to the University of Colorado Health Sciences Center. With 3600 deliveries per year, and thirty-six University residents rotating through the department annually, Rose is the major private teaching affiliate of the University.

Responsibilities would include private consultation, physician, resident, and nursing education, clinical research, and a limited private practice in perinatology. This individual can expect frequent interaction with the Maternal/Fetal Medicine division at the University of Colorado and would be eligible for an appropriate clinical appointment at the University.

A competitive salary guarantee is being offered with excellent fringe benefits including malpractice insurance.

Qualified applicants, including individuals completing fellowship in June, 1987 should forward CV's to:
Robert E. Wall, M.D.
Chairman, Department of OB/GYN
Rose Medical Center
4567 E. Ninth Avenue
Denver, Colorado 80220

Subspecialist Positions

615-bed tertiary care facility is seeking several board eligible or board certified subspecialists for the following areas within an expanding Department of Obstetrics and Gynecology.

- **Maternal/Fetal Medicine**
  Two new positions available in our established division of Maternal/Fetal Medicine. Ultrasound experience desirable.
- **Reproductive Endocrinology**
  An exceptional growth opportunity is available for a physician to direct this new division.

These positions offer excellent opportunities for professional development to qualified physicians interested in subspecialty practice, resident training, and research/faculty status in a large university affiliated hospital.

SINAI HOSPITAL OF DETROIT

Please submit your Curriculum Vitae or contact **Dr. David B. Schwartz, Chairman, Department of Obstetrics and Gynecology, Sinai Hospital of Detroit, 6767 W. Outer Drive, Detroit, MI 48235, or call (313) 493-5220.**

OBSTETRICIAN GYNECOLOGIST

Position available with The Permanente Medical Group, Inc., San Jose, California. Sixteen member OB/GYN Department seeking Board Certified/Eligible physicians for immediate openings. Excellent professional environment, competitive salary, outstanding benefits. Located in the San Francisco Bay Area, with superb educational, cultural and recreational activities.

Contact:

Jerome L. Nehemiah, M.D., Chief
Department of OB/GYN
The Permanente Medical Group, Inc.
260 International Circle
San Jose, CA 95119

Faculty Positions Available

University of Wisconsin, Milwaukee Clinical Campus, is seeking two full-time board eligible or board certified physicians for July, 1987.

PERINATOLOGIST: Position includes patient care and teaching with opportunities for basic or clinical research.

REPRODUCTIVE ENDOCRINOLOGIST: Position involves patient care and teaching with opportunities for research. Administrative role includes direction of *in vitro* fertilization program.

Send curriculum vitae to: Phillip R. Hamilton, M.D., Chairman, Department of Obstetrics and Gynecology, Mount Sinai Medical Center, 950 North 12th Street, Milwaukee, Wisconsin 53233.

GYNECOLOGIC ONCOLOGIST

The Division of Gynecologic-Oncology, Department of Obstetrics-Gynecology of the New York Hospital-Cornell University Medical Center is seeking a Board Eligible or Board Certified gynecologic-oncologist for a full time faculty position.

Interested persons please send CV to:

Thomas A. Caputo, M.D.
Director Gynecologic Oncology
Department of Obstetrics-Gynecology
The New York Hospital-
Cornell University Medical Center
525 East 68th Street
New York, New York 10021

An equal opportunity employer.

FACULTY POSITION AVAILABLE

The Department of Obstetrics and Gynecology at the Medical College of Ohio is accepting applications for a full-time position as director of the residency training program and generalist in the field of Obstetrics and Gynecology.

The residency director is responsible for administration of the program and patient care, including a busy clinical service along with resident and student teaching. Salary and academic rank will be commensurate with candidate's training and experience. Please direct inquiries and curricula vitae to:

John L. Duhring, M.D.
Chairman and Professor
Department of Obstetrics-Gynecology
Medical College of Ohio
C.S. 10008
Toledo, Ohio 43699
(419) 381-4591

ANNOUNCING THE
FIRST INTERNATIONAL SYMPOSIUM ON INTRAPARTUM BIOCHEMICAL MONITORING OF THE FETUS
JUNE 1-3, 1987
AT CAESAR'S CASINO AND HOTEL, ATLANTIC CITY, N.J.

This course will provide an update on intrapartum monitoring of fetus by biochemical means. The faculty includes world renowned authorities in the field. The course is designed to permit open question and answer sessions daily. It would also permit participants to address their particular problem to faculty person and other attendees during the Workshop and Free Communication Sessions. Special social program has been arranged for significant others accompanying the participants.

Physicians Fees:
$350
Resident Physicians:
$150
Medical Students, Nurses and Technicians:
$100
Deadline on Free Communication, March 28, 1987.
For further information contact:
Molly Chatterjee, M.D.
Our Lady of Mercy Medical Center
600 East 233rd Street
Bronx, New York 10466
Tel. (212) 920-9647

POSTGRADUATE FELLOWSHIP

MATERNAL-FETAL MEDICINE

Applications are invited for a two-year Fellowship in Maternal-Fetal Medicine under the direction of the Section of Maternal-Fetal Medicine, Department of Obstetrics, Gynecology and Reproductive Sciences, University of Manitoba, Dr. F.A. Manning, Professor. This position is available July 1, 1987.

The successful applicant will receive a comprehensive training experience in all aspects of high-risk obstetrics. Specific instruction in the real-time ultrasound assessment of the fetus using the biophysical profile score, intrauterine transfusion, fetal surgery, and experimental fetal research are an integral part of the program.

Applicants to this fully-funded program should possess Royal College of Physicians and Surgeons certification in Obstetrics and Gynecology or its equivalent. Applications including full C.V. and three professional references should be sent to:

C.R. Harman, M.D., Director
Fellowship Program
Section of Maternal-Fetal Medicine
University of Manitoba
735 Notre Dame Avenue
Winnipeg, Manitoba, MB. R3E OL8
Canada



The Johns Hopkins Oncology Center
Presents

The 13th Annual Symposium on
Diagnosis and Treatment of
Neoplastic Disorders
Management of Cancer in Women

April 2-3, 1987 at
The Johns Hopkins Medical Institutions
Baltimore, Maryland

14 AMA Category I Credits $250.00

For further information:
Office of Continuing Education
The Johns Hopkins Medical Institutions
Turner 22, 720 Rutland Avenue
Baltimore, Maryland 21205
(301) 955-6046

GYNECOLOGIC ONCOLOGIST

The Department of Gynecology at The University of Texas, M. D. Anderson Hospital and Tumor Institute at Houston is seeking a Board eligible/certified gynecologic oncologist at the Assistant Professor level. The candidate should have a strong commitment to academic medicine, teaching, and research. Salary will be commensurate with the candidate's training and experience. Please direct inquiries and curriculum vitae to:

J. Taylor Wharton, M.D.
Chairman
Department of Gynecology
MDAH Box 67
1515 Holcombe Blvd.
Houston, Texas 77030

An Equal Opportunity/Affirmative Action Employer

INDEX TO ADVERTISERS

Allentown Hospital, The
Opportunity Available 39A
American Society for Laser Medicine and Surgery
Seventh Annual Meeting 50A
Ayerst Laboratories
Premarin 22A,23A,24A
Ciba Pharmaceutical Company
Estraderm 43A,44A,45A,46A
Berkshire Medical Center
Opportunity Available 55A
Berlex Laboratories, Inc.
Tri-Levlen 52A,53A,54A
Group Health, Inc.
Opportunity Available 55A
Harvard Community Health Plan
Opportunity Available 55A
Health Resource Services
Opportunity Available 56A
Johns Hopkins Medical Institutions, The
Symposium 59A
Kaiser Permanente
Opportunity Available 55A
Mary Imogene Bassett Hospital
Opportunity Available 56A
Massachusetts General Hospital
Opportunity Available 56A
Mead Johnson Laboratories
Estrace 34A,35A,36A
Medical College of Ohio
Opportunity Available 57A,58A
Medical Equipment Designs, Inc.
HemoCue 10A,11A
Merck Sharp & Dohme
Mefoxin 48A,49A,50A
Michael Reese Health Plan
Opportunity Available 57A
Miles Pharmaceuticals
Mycelex Twin Pack 7A,8A
New York Hospital-Cornell University Medical Center
Opportunity Available 58A
Ortho Pharmaceutical Corporation
Ortho-Novum 7/7/7 ... Third Cover, Forth Cover
Our Lady of Mercy Medical Center
Opportunity Available 59A
Permanente Medical Group, Inc., The
Opportunity Available 58A
Postgraduate Obstetrics & Gynecology
Journal 47A
Rose Medical Center
Opportunity Available 57A
Saint John Hospital
Opportunity Available 39A
Serono Symposia, USA
Workshop 33A
Sinai Hospital of Detroit
Opportunity Available 57A
Trabin, Manko, & Litt, Drs.
Opportunity Available 39A
Smith Kline & French Laboratories
Cefizox 30A,31A,32A
Stuart Pharmaceuticals
Cefotan 40A,41A,42A
Syntex Laboratories, Inc.
Femstat 13A,15A,17A,18A
Tri-Norinyl ..... Second Cover,1A,2A
Trover Clinic
Opportunity Available 37A
UCLA School of Medicine
Opportunity Available 37A
University of Illinois College of Medicine
Opportunity Available 38A
University of Manitoba
Fellowship 59A
University of Minnesota
Opportunity Available 38A
University of Nebraska, The
Opportunity Available 38A
University of Tennessee Medical Center at Knoxville
Opportunity Available 38A
University of Texas, The
Opportunity Available 59A
University of Texas Health Science Center
Opportunity Available 37A
Virginia Baptist Hospital
Opportunity Available 37A
University of Wisconsin, Milwaukee Clinical Campus
Opportunity Available 58A
Upjohn Company
Provera 3A,4A

While every precaution is taken to insure accuracy, we cannot guarantee against the possibility of an occasional change or omission in the preparation of this index.

ORTHO-NOVUM 1/77

TRADEMARK

(norethindrone/ethinyl estradiol)

Tablets

Unique, week-by-week design

Each white tablet contains 0.5 mg norethindrone and 0.035 mg ethinyl estradiol. Each light peach tablet contains 0.75 mg norethindrone and 0.035 mg ethinyl estradiol. Each peach tablet contains 1 mg norethindrone and 0.035 mg ethinyl estradiol. Each green tablet in 28-day regimen contains inert ingredients.

IMPORTANT NOTE--This information is a BRIEF SUMMARY of the complete prescribing information provided with the product and therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables, and references. The physician should be thoroughly familiar with the complete prescribing information before prescribing the product.

INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

DOSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing .05 mg or less of estrogen.

CONTRAINDICATIONS: Oral contraceptives should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed, abnormal genital bleeding. 7. Oral contraceptive tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who are pregnant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study conducted in the United States found that past use of oral contraceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk may be related to duration of oral contraceptive use. **MYOCARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor in determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified, it is quite likely that the same synergistic action exists, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists following discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the past use. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34 -- 5/100,000; ages 35-44 -- 33/100,000; ages 45-49 -- 140/100,000); the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is discontinued. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over especially those with other risk factors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. OCULAR LESIONS. There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual partial or complete loss of vision, onset of proptosis or diplopia, papilledema, or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. CARCINOMA. Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. HEPATIC TUMORS. Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5. USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING. The use of female sex hormones--both estrogenic and progestational agents--during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. GALLBLADDER DISEASE. Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. HEADACHE. The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe requires discontinuation of oral contraceptives and evaluation of the cause. 10. BLEEDING IRREGULARITIES. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. BREAST FEEDING. Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General.** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **INFORMATION FOR THE PATIENT: (See Patient Package Insert).**

DRUG INTERACTIONS: Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **CARCINOGENESIS, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS and WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea, usually the most common adverse reaction. Vomiting, occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Revised 12/84

ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869

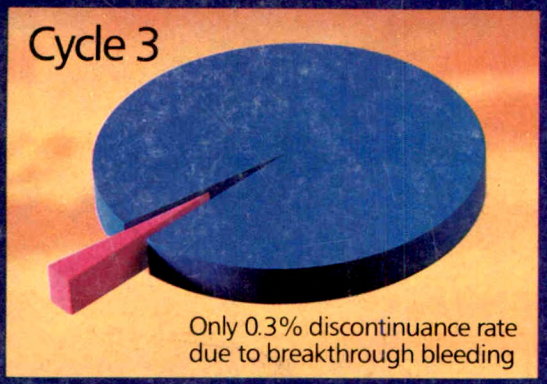
OJ-781

ORTHO

#1 for new start patien

What to look for in a phasic†...Reason 2

Low breakthrough bleeding



Only 0.3% discontinuance rate due to breakthrough bleeding

- ☐ High rates of patient acceptance
- ☐ Endometrial support throughout the cycle

7 7 7

Designed for performance

21- and 28-day regimens

ORTHO-NOVUM 7/7/7 Tablets

TRADEMARK

(norethindrone/ethinyl estradiol)

†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

Please see complete Prescribing Information, a summary of which appears on the preceding page.

ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869

OJ-782R

© OPC 1985

ORTHO

March 1987
volume 156, *number* 3

VAMC Loma Linda, Hosp. Lib.

American Journal of OBSTETRICS AND GYNECOLOGY

Copyright © 1987 by The C. V. Mosby Company

Editor in Chief
JOHN I. BREWER

Editors
FREDERICK P. ZUSPAN • E. J. QUILLIGAN

Associate Editor
ALBERT B. GERBIE

Official Publication

AMERICAN GYNECOLOGICAL AND OBSTETRICAL SOCIETY
CENTRAL ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
SOCIETY OF OBSTETRICIANS AND GYNAECOLOGISTS OF CANADA
SOUTH ATLANTIC ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
PACIFIC COAST OBSTETRICAL AND GYNECOLOGICAL SOCIETY
AMERICAN BOARD OF OBSTETRICS AND GYNECOLOGY
SOCIETY FOR GYNECOLOGIC INVESTIGATION
SOCIETY OF PERINATAL OBSTETRICIANS

Published by
THE C. V. MOSBY COMPANY
St. Louis, Missouri 63146

ISSN 0002-9378

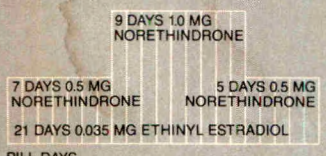


UNIQUE

Full-cycle endometrial support, with no late-cycle progestin increase.



The unique TRI-NORINYL phasing achieves BTB control equivalent to a 1/35 formulation, but with 29% less progestin per cycle. This is, in fact, the greatest progestin reduction of any norethindrone-based multiphasic. TRI-NORINYL lowers progestin where requirements for endometrial support are reduced—with no greater reported BTB than a 1/35 formulation.*



SUPPLIED IN THE EXCLUSIVE SYNTEX WALLETTE™ PILL DISPENSER



Preferred by patients 3 to 1 over other available packages for convenience, discreetness and compliance.**

THE NATURE OF TRI-NORINYL®

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

*1/35 formulations contain 1.0 mg norethindrone with 0.035 mg ethinyl estradiol. Data available from Syntex Laboratories, Inc.

**Based on a nationwide, independently conducted survey of prospective oral contraceptive patients. Data available from Syntex Laboratories, Inc.

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see brief summary of full prescribing information on next page.

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

©1986 Syntex Laboratories, Inc.

THE NATURE OF TRI-NORINYL®

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

TRI-NORINYL® 21-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)

TRI-NORINYL® 28-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg; 7 inert tablets)

ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications Prevention of pregnancy. *DOSE-RELATED RISK OF THROMBOEMBOLISM.* Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested the persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34 – 5/100,000; ages 35-44 – 33/100,000; ages 45-49 – 140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34 – 1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over – 1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and for MI is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS – RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40 +
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A – Use associated with very high risk.
B – Use associated with high risk.
C – Use associated with moderate risk.
D – Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden, or gradual, partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (31-13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to the registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer, and other studies suggested an increased risk of breast cancer with long-term OC use before age 25. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared to nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. Two other studies suggested an increasing incidence of cervical neoplasms with longer duration of OC use. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OCs with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones – estrogenic and progestational agents – during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase the risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in the results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. One study suggests *Chlamydia trachomatis* infection, the leading cause of nongonococcal P.I.D., is enhanced by OCs. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failure. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions** *GENERAL* 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding from interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, antimigraine preparations, antihistamines, and griseofulvin. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, theophylline, and beta blockers. Toxic liver effects have been reported with a combination of an OC and cyclosporin A. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Warnings. **Adverse Reactions** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps, colitis), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued; breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, changes in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution of lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chilblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, vaginitis, porphyria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome), malignant hypertension, acute renal failure. **Information for the Patient** (See Patient Package Insert).

Rev. 6/86

SYNTEX

SYNTEX LABORATORIES, INC
PALO ALTO, CA 94304

A Standard in Progesterone Therapy

Provera® 10 MG TABLETS
(medroxyprogesterone acetate)

WARNING

THE USE OF PROGESTATIONAL AGENTS DURING THE FIRST FOUR MONTHS OF PREGNANCY IS NOT RECOMMENDED.

Progestational agents have been used beginning with the first trimester of pregnancy in an attempt to prevent habitual abortion or treat threatened abortion. There is no adequate evidence that such use is effective and there is evidence of potential harm to the fetus when such drugs are given during the first four months of pregnancy. Furthermore, in the vast majority of women, the cause of abortion is a defective ovum, which progestational agents could not be expected to influence. In addition, the use of progestational agents, with their uterine-relaxant properties, in patients with fertilized defective ova may cause a delay in spontaneous abortion. Therefore, the use of such drugs during the first four months of pregnancy is not recommended.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies including congenital heart defects and limb reduction defects. One study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000.

If the patient is exposed to PROVERA Tablets (medroxyprogesterone acetate) during the first four months of pregnancy or if she becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus.

INDICATIONS: Secondary amenorrhea; abnormal uterine bleeding due to hormonal imbalance in the absence of organic pathology such as fibroids or uterine cancer.

CONTRAINDICATIONS: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy or patients with a past history of these conditions. Liver dysfunction or disease. Known or suspected malignancy of breast or genital organs. Undiagnosed vaginal bleeding. Missed abortion. Known sensitivity to medroxyprogesterone acetate. As a diagnostic test to pregnancy.

WARNINGS: 1. Immediately discontinue administration should any of the following thrombotic disorders occur or be suspected: thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis. **2.** Beagle dogs treated with medroxyprogesterone acetate developed mammary nodules some of which were malignant. Although nodules occasionally appeared in control animals, they were intermittent in nature; whereas the nodules in the drug treated animals were larger, more numerous, persistent, and there were some breast malignancies with metastases. Their significance with respect to humans has not been established. **3.** Discontinue medication pending examination if there is sudden partial or complete loss of vision, onset of proptosis, diplopia, or migraine. If papilledema or retinal vascular lesions occur, withdraw medication. **4.** Detectable amounts of progestin have been identified in the milk of mothers receiving the drug. The effect of this on the nursing infant has not been determined. **5.** Usage in pregnancy is not recommended (see Warning box). **6.** Three major studies in Great Britain and one in this country have shown a statistically significant association between thrombophlebitis, pulmonary embolism, cerebral thrombosis and embolism and the use of oral contraceptives. It has been estimated that users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study indicated that the risk did not persist after discontinuation, and it was not enhanced by long-continued administration.

PRECAUTIONS: A pretreatment physical exam should include special reference to breast and pelvic organs and a Papanicolaou smear. This drug may cause fluid retention; therefore, carefully observe patients with conditions influenced by fluid retention such as epilepsy, migraine, asthma, and cardiac or renal dysfunction. In irregular bleeding per vaginum, bear in mind nonfunctional causes and perform adequate diagnostic measures. Advise pathologist of therapy when submitting relevant specimens. Carefully observe patients with history of psychic depression and discontinue drug if serious depression recurs. Any possible influence of prolonged therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study. Decreased glucose tolerance has been observed in a small percentage of patients on estrogen-progestin combinations; therefore, carefully observe diabetic patients receiving progestin therapy. Age constitutes no absolute limiting factor, although onset of climacteric may be masked. Because of the occasional occurrence of thrombotic disorders (thrombophlebitis, pulmonary embolism, retinal thrombosis, and cerebrovascular disorders) in patients taking estrogen-progestin combinations and since the mechanism is obscure, the physician should be alert to the earliest manifestation of these disorders. (See Patient Information for complete prescribing information.)

ADVERSE REACTIONS: Pregnancy: (see Warning box); **Breast:** rare reports of breast tenderness or galactorrhea; **Skin:** sensitivity reactions including pruritus, urticaria, edema and generalized rash; acne, alopecia and hirsutism in a few patients; **Thromboembolic Phenomena** including thrombophlebitis and pulmonary embolism.

The following adverse reactions have been observed in women taking progestins including medroxyprogesterone acetate: breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; edema; change in weight; changes in cervical erosion and secretions; cholestatic jaundice; rash (allergic) with and without pruritus; mental depression; anaphylaxis and anaphylactoid reactions; pyrexia; insomnia; nausea and somnolence.

A statistically significant association has been demonstrated between use of estrogen-progestin combination drugs and the serious adverse reactions of thrombophlebitis, pulmonary embolism and cerebral thrombosis and embolism. Therefore, patients on progestin therapy should be carefully observed.

Although available evidence is suggestive, a relationship has been neither confirmed nor refuted for the association of the serious adverse reaction of neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been observed in patients receiving estrogen-progestin combination drugs: rise in blood pressure in susceptible individuals; premenstrual-like syndrome; changes in libido; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; fatigue; backache; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; and itching.Therefore, observe patients on progestin therapy carefully.

The following laboratory results may be altered by the use of estrogen-progestin combination drugs: increased sulfobromophthalein retention and other hepatic function tests; coagulation tests (increase in prothrombin factors VII, VIII, IX and X); metyrapone test; pregnanediol determination; thyroid function tests (increase in PBI, and butanol extractable protein bound iodine and decrease in T^3 uptake values).

Caution: Federal law prohibits dispensing without prescription.

MED B-13-S

Upjohn

A Century of Caring
1886·1986

March 1986
J-6144

© 1986 The Upjohn Company

The Upjohn Company, Kalamazoo, MI 49001

A Standard



Provera® 10 MG TABLETS
(medroxyprogesterone acetate)

A Standard in Progesterone Therapy

Please see adjacent page for brief summary of prescribing information.

MOSBY

American Journal of Obstetrics and Gynecology

Founded in 1920

Copyright © 1987 by The C. V. Mosby Company

Contents

March
1987

EDITORIAL

Summary of analysis of published articles 525

CLINICAL SECTION

Clinical Opinion

The use and misuse of the fetal biophysical profile 527
Anthony M. Vintzileos, M.D., Winston A. Campbell, M.D., David J. Nochimson, M.D., and Paul J. Weinbaum, M.D.
Farmington, Connecticut

Maternal serum α-fetoprotein screening. II. Pitfalls in low-volume decentralized laboratory performance 533
James N. Macri, Ph.D., Ramana V. Kasturi, B.E., M.S., David A. Krantz, B.S., and Mannshya G. Hu, Ph.D.
Carle Place, New York

(Contents continued on page 6A)

Vol. 156, No. 3, March 1987. The American Journal of Obstetrics and Gynecology (ISSN 0002-9378) is published monthly by The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146. POSTMASTER: Send address changes to The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

1987 Annual subscription rates	U.S.A.	International (surface mail) All regions	International (airmail)* Canada & Mexico	Region 1	Region 2	Region 3
Institutions†	$102.00	$121.00	$142.55	$153.60	$183.85	$214.10
Individuals‡	$ 59.50	$ 78.50	$100.05	$111.10	$141.35	$171.60
Students, residents‡	$ 40.00	$ 59.00	$ 80.55	$ 91.60	$121.85	$152.10

Single copies are $6.50. Remittances should be made by check, draft, or post office or express money order, in U.S. funds drawn through a U.S. bank, payable to this JOURNAL. Claims for missing issues will be serviced only within six months of cover date. Single copy prices will be charged on missing issue claims older than six months from cover date.

*Airmail breakdown—Domestic: First-class and Priority rates for the U.S. and possessions are available upon request. *Region 1:* Central America, islands, and mainland colonies of European countries in The Americas. *Region 2:* South America, Europe, Egypt, Africa (bordering the Mediterranean). *Region 3:* Asia, Australasia, Africa (other than Mediterranean), Middle East, Far East, The Pacific, U.S.S.R. (and constituent Republics).

†Institutional (multiple-reader) subscriptions are available to public and private libraries, schools, hospitals, and clinics; city, county, state, provincial, and national government bureaus and departments; and all commercial and private institutions and organizations.

‡Individual subscriptions and all student-rate subscriptions must be in the names of, billed to, and paid by individuals. All student-rate requests must indicate training status and name of institution.

Subscriptions may begin at any time.

Second-class postage paid at St. Louis, Missouri, and additional mailing offices. Printed in the U.S.A. Copyright © 1987 by The C. V. Mosby Company.

Contents continued from page 5A

Clinical Articles

Prevention of preterm birth by ambulatory assessment of uterine activity: A randomized study 536
John C. Morrison, M.D., James N. Martin, Jr., M.D., Rick W. Martin, M.D., Kathy S. Gookin, R.N., M.S.N., and Winfred L. Wiser, M.D.
Jackson, Mississippi

Braxton Hicks' contractions and motor behavior in the near-term human fetus 543
E. J. H. Mulder, M.Sc., and G. H. A. Visser, M.D., Ph.D.
Groningen, The Netherlands

(Sub)Specialization in obstetrics and gynecology: Results of a survey by The American Board of Obstetrics and Gynecology 550
James A. Merrill, M.D.
Seattle, Washington

The diagnostic value of hysterosalpingography and hysteroscopy in infertility investigation 558
Jamil A. Fayez, M.D., Ghazi Mutie, M.D., and Peter J. Schneider, M.D.
Winston-Salem, North Carolina

Treatment of menopausal hot flashes with transdermal administration of clonidine 561
Manubai Nagamani, M.D., Mae Ellen Kelver, M.D., and Edward R. Smith, Ph.D.
Galveston, Texas

Vaginal birth after two or more previous cesarean sections 565
George Farmakides, M.D., Roger Duvivier, M.D., Harold Schulman, M.D., Elizabeth Schneider, M.D., and John Biordi, M.D.
Mineola, New York

Prospective pricing system by diagnosis-related groups: Comparison of federal diagnosis-related groups with high-risk obstetric care groups 567
Michael B. Resnick, Ed.D., Mario Ariet, Ph.D., Randolph L. Carter, Ph.D., Andres Cao, M.B.A., Robert R. Furlough, Ph.D., Janet H. Evans, M.S.P.A., Allan G. W. McLeod, M.D., Amelia C. Cruz, M.D., Richard L. Bucciarelli, M.D., John S. Curran, M.D., and William W. Ausbon, M.D.
Gainesville, Tallahassee, Miami, and Tampa, Florida

Fetal diaphragmatic hernia: Ultrasound diagnosis and clinical outcome in 19 cases 573
Beryl R. Benacerraf, M.D., and N. Scott Adzick, M.D.
Boston, Massachusetts

(Contents continued on page 9A)

Mycelex® Twin Pack
(clotrimazole)

One-day therapy

Mycelex®-G 500 mg
(clotrimazole) Vaginal Tablet

The shortest regimen for vulvovaginal candidiasis

In the case of severe vulvovaginitis due to candidiasis, longer antimycotic therapy is recommended.

Please see following page for brief summary of prescribing information.

Seven-day therapy

Mycelex® 1%
(clotrimazole) Cream

For prompt relief of cutaneous symptoms



Mycelex® Twin Pack

Miles Pharmaceuticals
Division of Miles Laboratories, Inc.
West Haven, Connecticut 06516 USA

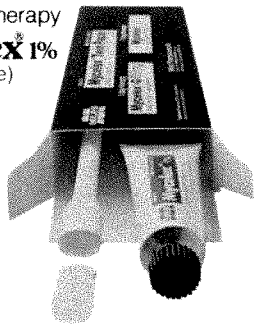
© 1986 Miles Pharmaceuticals Division of Miles Laboratories, Inc.

MILES
Miles Pharmaceuticals

Mycelex® Twin Pack
(clotrimazole)

One-day therapy
Mycelex®-G 500mg
(clotrimazole) Vaginal Tablet

Seven-day therapy
Mycelex® 1%
(clotrimazole)
Cream



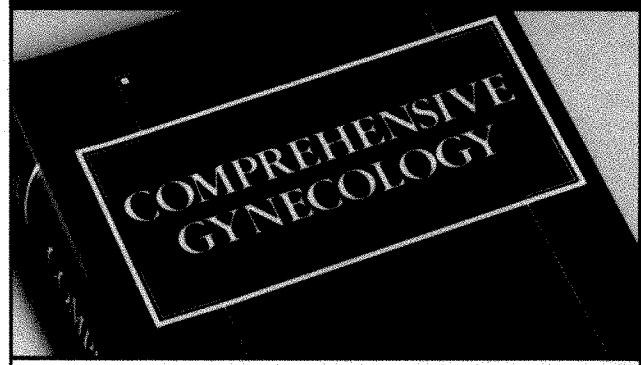
Indications
MYCELEX®-G 500 mg Vaginal Tablets are indicated for the local treatment of vulvovaginal candidiasis when one-day therapy is felt warranted. In the case of severe vulvovaginitis due to candidiasis, longer antimycotic therapy is recommended.
MYCELEX® CREAM is indicated for the topical treatment of dermal infections due to *Candida albicans*.
Contraindications
MYCELEX-G 500 mg Vaginal Tablets and MYCELEX CREAM are contraindicated in women who have shown hypersensitivity to any of their components.
Warnings
MYCELEX-G 500 mg Vaginal Tablets: none. MYCELEX CREAM is not for ophthalmic use.
Precautions
MYCELEX-G 500 mg Vaginal Tablets: If there is a lack of response to the Vaginal Tablets, appropriate microbiological studies should be repeated to confirm the diagnosis and rule out other pathogens before instituting another course of antimycotic therapy.
MYCELEX CREAM: If irritation or sensitivity develops with the use of Mycelex Cream, treatment should be discontinued and appropriate therapy instituted.
Carcinogenesis
MYCELEX-G 500 mg Vaginal Tablets: No long-term studies in animals have been performed to evaluate the carcinogenic potential of 500 mg Vaginal Tablets intravaginally. A long-term study in rats (Wistar strains) where clotrimazole was administered orally provided no indication of carcinogenicity.
Usage in Pregnancy: Pregnancy Category B:
MYCELEX-G 500 mg Vaginal Tablets: The disposition of ^{14}C-clotrimazole has been studied in humans and animals. Clotrimazole is poorly absorbed following intravaginal administration to humans, whereas it is rather well absorbed after oral administration.
In clinical trials, use of vaginally applied clotrimazole in pregnant women in their second and third trimesters has not been associated with ill effects. There are, however, no adequate and well-controlled studies in pregnant women during the first trimester of pregnancy. Because animal reproduction studies are not always predictive of human response, this drug should be used only if clearly indicated during the first trimester of pregnancy.
MYCELEX CREAM: In the first trimester of pregnancy, Mycelex Cream should be used only when considered essential to the welfare of the patient.
Adverse Reactions
MYCELEX-G 500 mg Vaginal Tablets: Of 297 patients in double-blind studies with the 500 mg vaginal tablet, 3 of 149 patients treated with active drug and 3 of 148 patients treated with placebo reported complaints during therapy that were possibly drug related. In the active drug group, vomiting occurred in one patient, vaginal soreness with coitus in another, and complaints of vaginal irritation, itching, burning and dyspareunia in the third patient. In the placebo group, clitoral irritation occurred in one patient and dysuria, described as remotely related to drug, in the other. A third patient in the placebo group developed bacterial vaginitis which the investigator classed as possibly related to drug.
MYCELEX CREAM: The following reactions have been reported in connection with the use of this cream: erythema, stinging, blistering, peeling, edema, pruritus, urticaria, and general irritation of the skin.

Issued, July 1986

Miles Pharmaceuticals
Division of Miles Laboratories, Inc.
West Haven, Connecticut 06516 USA

MILES
Miles Pharmaceuticals

Innovation Plus Expertise!



COMPREHENSIVE GYNECOLOGY

By William Droegemueller, M.D.; Arthur L. Herbst, M.D.; Daniel R. Mishell, Jr., M.D.; and Morton A. Stenchever, M.D.

Innovative enough to emphasize the psychosocial treatment of your patient, with the expertise to cover a full range of diseases and physical problems — this combination brings you the first, all new comprehensive gynecology text in years! Comprehensive discussions on:

- the most current treatments available
- psychosocial approaches to the troubled patient
- family planning and pregnancy termination
- breast disease and sexually transmitted diseases, including AIDS in women
- rape, incest and other physical abuse

The Key Terms and Definitions that begin each chapter and the synopsis of Key Points at the end provide a quick review. Sophisticated graphics highlight pertinent data. Written by four well-known specialists who share their experience and expertise, COMPREHENSIVE GYNECOLOGY presents state-of-the-art information you'll refer to again and again.

July, 1987. Approx. 1,000 pages with 800 illustrations. (Book Code: 01929) About $60.00 (U.S.); $82.95 (Can.)

In a hurry to order?
Call FREE 800-221-7700, ext. 15A.
Our hotline is open 24 hours a day, 7 days a week. In Canada call 416-298-1588.
AMS-969-031

MOSBY
TIMES MIRROR

Yes! Send me a copy of Droegemueller's COMPREHENSIVE GYNECOLOGY (01929) on 30-day approval priced about $60.00 (U.S.); $82.95 (Can.)
Save money! Enclose payment with your order or charge it to your credit card. Mosby will pick up the tab for shipping and handling.
☐ Payment enclosed
Charge my credit card: ☐ MasterCard ☐ VISA
Card # _____ Exp. date _____
Signature _____
Business phone (_____) _____
☐ Bill me, plus shipping and handling.
Name _____
Address _____
City _____
State _____ Zip _____
☐ Add me to your mailing list. (No purchase necessary)
Specialty _____

To properly process Purchase Orders and for more personal service, mail this coupon to: Dennis Carson, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, MO 63146. 30-day approval good in U.S. and Canada. Outside U.S. and Canada, please pay in dollar equivalent. ALL PRICES SUBJECT TO CHANGE. Add applicable sales tax.

AMS-969-031-03

Contents continued from page 6A

A viable pregnancy in a patient with myositis ossificans progressiva 577
Yvonne S. Thornton, M.D., Stanley J. Birnbaum, M.D., and Nancy Lebowitz, M.D.
New York, New York

Can ultrasonography replace amniocentesis in fetal gender determination during the early second trimester? 579
E. Albert Reece, M.D., Hung N. Winn, M.D., Macor Wan, M.D., Carol Burdine, R.D.M.S., Jacqueline Green, M.S., R.D.M.S., and John C. Hobbins, M.D.
New Haven, Connecticut

Atraumatic removal of secretions from the prepubertal vagina 581
Susan F. Pokorny, M.D., and Jane Stormer, L.V.N.
Houston, Texas

Preliminary report on the use of tamoxifen in the treatment of endometriosis 582
G. M. Haber, M.D., and Y. F. Behelak, Ph.D., M.D.
Montreal, Quebec, Canada

Exaggerated gonadotropin response to luteinizing hormone–releasing hormone in amenorrheic runners 586
Janet Yahiro, Ph.D., Allan R. Glass, M.D., William B. Fears, M.D., Earl W. Ferguson, M.D., Ph.D., and Robert A. Vigersky, M.D.
Bethesda, Maryland, and Washington, D. C.

The effect of low-dose oral contraceptives on cardiorespiratory function, coagulation, and lipids in exercising young women: A preliminary report 591
Morris Notelovitz, M.D., Ph.D., Chris Zauner, Ph.D., Lynda McKenzie, R.N., M.Ed., Yvonne Suggs, R.N., Carol Fields, M.P.E., and Craig Kitchens, M.D.
Gainesville and Miami, Florida

Reassessment of White's classification and Pedersen's prognostically bad signs of diabetic pregnancies in insulin-dependent diabetic pregnancies 599
Michael P. Diamond, M.D., Sheron L. Salyer, R.N.C., William K. Vaughn, Ph.D., Robert Cotton, M.D., and Frank H. Boehm, M.D.
Nashville, Tennessee

Legal abortion mortality in the United States: 1972 to 1982 605
Hani K. Atrash, M.D., H. Trent MacKay, M.D., Nancy J. Binkin, M.D., and Carol J. R. Hogue, Ph.D.
Atlanta, Georgia

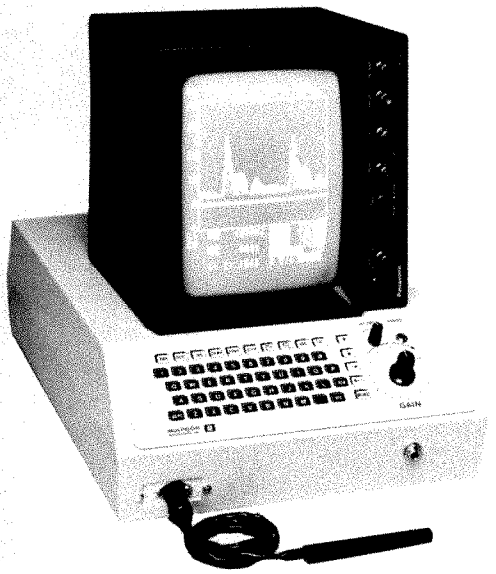
(Contents continued on page 11A)

MULTIGON INDUSTRIES, INC.

500A™ UMBILICAL-UTERINE ARTERY DOPPLER SPECTRUM ANALYZER

LETS YOU EVALUATE

UTEROPLACENTAL PERFUSION BY SPECTRUM ANALYSIS OF UMBILICAL AND MATERNAL UTERINE ARTERY DOPPLER WAVEFORMS

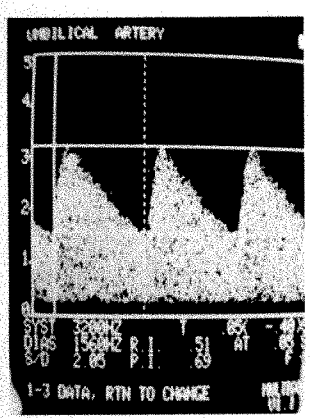


CLINICAL RESULTS[1-8] SHOW THAT YOU CAN:

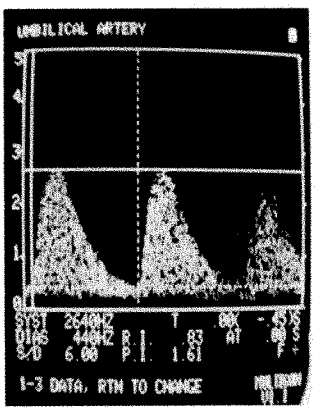
- DETECT *IUGR* FROM 20 WEEKS IN SINGLETON PREGNANCIES AND 30 WEEKS IN TWIN PREGNANCIES
- USE UTERINE ARTERY WAVEFORMS IN THE MANAGEMENT OF MATERNAL PREECLAMPSIA
- USE UMBILICAL ARTERY VELOCITY WAVEFORMS IN THE MANAGEMENT OF DIABETIC PREGNANCIES

Umbilical and uterine artery waveforms are easily obtained from early pregnancy on. Low power continuous wave Doppler spectrum analysis is used in a simple test to measure the peripheral resistance in the placental circulation. This resistance is measured by taking the ratio of the systolic to diastolic (S/D) velocity. The S/D ratio is compared to established normal values to determine the presence of abnormal resistance.

The Model 500A™ automatically computes the systolic to diastolic ratio as well as resistance and pulsatility indices.



Normal Umbilical Waveform
S/D = 2.05



Abnormal Umbilical Waveform
S/D = 6.00

REFERENCES

1. Schulman H, Fleischer A, Stern W, *Jagani N, Farmakides G, Blattner P. Umbilical artery velocity waveforms in human pregnancy.* AM J OBSTET GYNECOL 1984;148:985
2. Campbell, Griffin, Pearce, *Diaz-Recasens, Cohen-Overbeek, Wilson and Teague.* The Lancet, March 1983, pp. 675-677.
3. Giles, WB, Trudinger BJ, Baird PJ: *Fetal umbilical artery flow velocity waveforms and placental resistance: pathological correlation.* Br J Obstet Gynaec 92:31-38, 1985
4. Farmadikes G, Schulman B, Seldana LR, *Bracero LA, Fleischer A, Rochelson B: Surveillance of twin pregnancy with umbilical arterial velocimetry:* Am J Obstet Gynecol 153:789-792, 1985
5. Trudinger, Giles and Cook. Brit. J. Obs. Gyn. Jan. 1985, Vol. 92, pp. 39-45.
6. Fleischer A, Schulman B, Farmadikes G, *Bracero L, Blattner F, Randolph G: Umbilical artery velocity waveforms and interuterine growth retardation.* Am J. Obstet Gynecol 151:502-505, 1985
7. Trudinger, Giles, Cook, *Bombardieri and Collins.* Brit. J. Obs. Gyn. Jan. 1985, Vol. 92, pp. 22-30.
8. Fleischer A, Schulman H, Farmadikes G, *Bracero L, Grunfeld L, Rochelson B, Koenigsberg M: Uterine artery Doppler Velocimetry in pregnant women with hypertension.* Am J Obstet Gynecol 154:806-814, 1986

MULTIGON INDUSTRIES, INC.
P.O. BOX 2485 • MT. VERNON, N.Y. 10551
(914) 664-7300 Telex 6974662 MULTIGN

Contents continued from page 9A

Successful pregnancy outcome after cervical dilation with multiple laminaria tents in preparation for second-trimester elective abortion: A report of two cases 612
Linda Van Le, M.D., and Philip D. Darney, M.D., M.Sc.
San Francisco, California

Long-term observations of cervical cancer 614
Axel N. Arneson, M.D., and Ming Shian Kao, M.D.
St. Louis, Missouri

The value of endocervical curettage in the management of the patient with abnormal cervical cytologic findings 625
David A. Grainger, M.D., Daniel K. Roberts, M.D., Ph.D., Max M. Wells, M.D., and Douglas V. Horbelt, M.D., M.A.
Wichita, Kansas

Atypia in cervical cytology as a risk factor for intraepithelial neoplasia 628
Joel S. Noumoff, M.D.
Philadelphia, Pennsylvania

Magnesium sulfate and ritodrine hydrochloride: A randomized comparison 631
David I. Hollander, M.D., David A. Nagey, M.D., Ph.D., and Marcos J. Pupkin, M.D.
Baltimore, Maryland

Treatment of acute intrapartum fetal distress by β_2-sympathomimetics 638
C. Mendez-Bauer, M.D., Ph.D., Afrasiab Shekarloo, M.D., Vernon Cook, M.D., and Uwe Freese, M.D.
Chicago, Illinois, Madrid, Spain, and Montevideo, Uruguay

Upper airway obstruction as a cause of pulmonary edema during late pregnancy 643
Urs Lagler, M.D., and Erich Russi, M.D.
Zurich, Switzerland

Symphysis-fundus height, maternal height, labor pattern, and mode of delivery 644
Alan B. Hughes, M.D., D. Adam Jenkins, M.B., B.S., B.Sc., Robert G. Newcombe, Ph.D., and James F. Pearson, M.D.
Cardiff, Wales

Preterm labor: Stimulation of arachidonic acid metabolism in human amnion cells by bacterial products 649
Phillip R. Bennett, B.Sc., M.B., B.S., Mathew P. Rose, Ph.D., Leslie Myatt, Ph.D., and Murdoch G. Elder, M.D.
London, England

(Contents continued on page 14A)

Accurate Hemoglobin test

The secret of the HemoCue Hb test system lies in the unique, disposable microcuvette, which serves as pipette, test tube and measuring vessel in one. Containing a dry reagent, it self-fills with blood by capillary action to produce a precise chemical reaction.



Finland: Oy Tamro Ab. Tel. 90-544 011. **Norway:** A/S Saba Medical. Tel. (02) 64 47 30. **Iceland:** O Olafsson h/f. Tel. 01-84166.
Italy: Labanalysis srl. Tel. (06) 45 03 918. **The Netherlands:** UCB Farma Nederla
Tel. 01-932 30 92. **Great Britain:** Clandon Scientific Ltd. Tel. (0252) 51 47 11/5. **Australia & New Zealand:** Medipac Pty Ltd.
Tel. 274 66 23. **Malaysia:** Translab (M) Sdn Bhd. Tel. 03-78 10 44. **Indonesia:** Berca Indonesia Pt. Tel. 021-34 38 86.
Tel. 63-43 74 42. **Taiwan:** Transmedic Co Ltd. Tel. 02-981 5343. **South Korea:** Poong Nam Corp. Tel. (02) 557-6947.

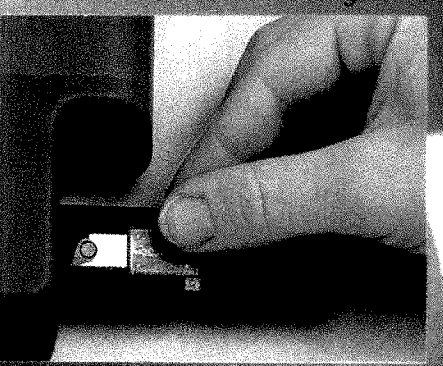
results within 60 seconds!



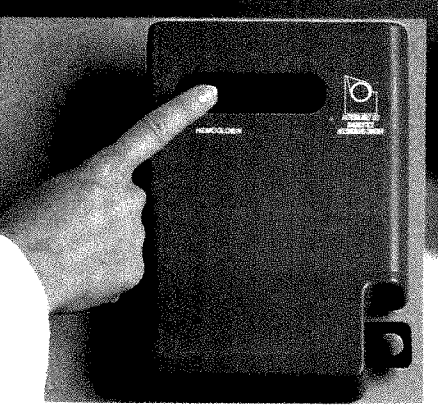
1 *Obtain a blood sample by finger stick.*



2 *The HemoCue microcuvette draws in an exact volume of blood.*



...cuvette into the



4 *Result displayed automatically within 15–45 seconds.*

...he HemoCue photometer is fully a... The only ...ntrol is the ON/OFF switch! Zero setting ... ...djustments are performed automatically betwe... ...also automatically compensates for turbidity in ...e sample!

Thanks to HemoCue you can now get **accurate** Hb test ...sults, truly stat, while the patient is still in your office.

For more information on this new and economical test, ...ll 800-323-1674 (CA 714-859-7779).

HemoCue®

...eveloped and manufactured by
...o Diagnostics AB, S-251 09 Helsingborg, Sweden.
...arketing and technical service in the USA exclusively through
...edical Equipment Designs, Inc.
...521 Ridge Route Dr. Suite A, Laguna Hills, CA 92653.

Contents continued from page 11A

Umbilical artery Doppler blood flow studies in the preterm, small for gestational age fetus 655
Lesley M. McCowan, M.B., Lawrence A. Erskine, M.B., and Knox Ritchie, M.D.
Toronto, Ontario, Canada

Adjuvant therapy for Stage I uterine sarcoma 660
Peter G. Rose, M.D., John G. Boutselis, M.D., and Larry Sachs, Ph.D.
Columbus, Ohio

Clinicopathologic study of eight cases of uterine body cancers associated with endometriosis interna (uterine adenomyosis) 663
Takashi Hayata, M.D., and Yoshiro Kawashima, M.D.
Hamamatsu, Japan

Type-specific streptococcal antibodies in amniotic fluid 666
Barry M. Gray, M.D., Janet D. Springfield, M.P.H., and Hugh C. Dillon, Jr., M.D.
Birmingham, Alabama

Luteal function in ectopic pregnancy 669
Corinne J. Hubinont, M.D., Christine Thomas, M.D., and Jean F. Schwers, M.D.
Brussels, Belgium

Pregnancy-induced changes in the fibrinolytic balance: Evidence for defective release of tissue plasminogen activator and increased levels of the fast-acting tissue plasminogen activator inhibitor 674
Margaret Gore, M.D., Susan Eldon, A.B., Kenneth F. Trofatter, M.D., Ph.D., Seng-Jaw Soong, Ph.D., and Salvatore V. Pizzo, M.D., Ph.D.
Durham, North Carolina, and Birmingham, Alabama

The effects of human chorionic gonadotropin on growth and steroidogenesis of the human fetal adrenal gland in vitro 681
Maysan Abu-Hakima, B.Sc., Charlotte L. Branchaud, Ph.D., Cynthia G. Goodyer, Ph.D., and Beverley E. Pearson Murphy, M.D., Ph.D.
Montreal, Quebec, Canada

Reliable criteria for the prenatal sonographic diagnosis of alobar holoprosencephaly 687
Michael F. Greene, M.D., Beryl R. Benacerraf, M.D., and Fredric D. Frigoletto, Jr., M.D.
Boston, Massachusetts

Fetal cardiac tumor: A prenatal echocardiographic marker for tuberous sclerosis 689
C. D. Gresser, R.N., R.D.M.S., J. Shime, M.D., H. Rakowski, M.D., J. F. Smallhorn, M.B., B.S., A. Hui, M.D., and J. J. Berg, M.B., M.Sc.
Toronto, Ontario, Canada

(Contents continued on page 16A)

Natalins® Rx

Multivitamin and Multimineral Supplement with 1 mg Folic Acid

The Only Prenatal Vitamin with No Artificial Color or Flavor from Synthetic Sources

The Recipe for Prenatal Good Health

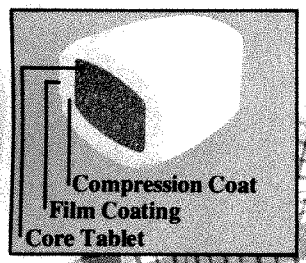
Good things are made from the finest ingredients. And the ingredients for maintaining good prenatal health make NATALINS Rx a standout . . . inside and out.

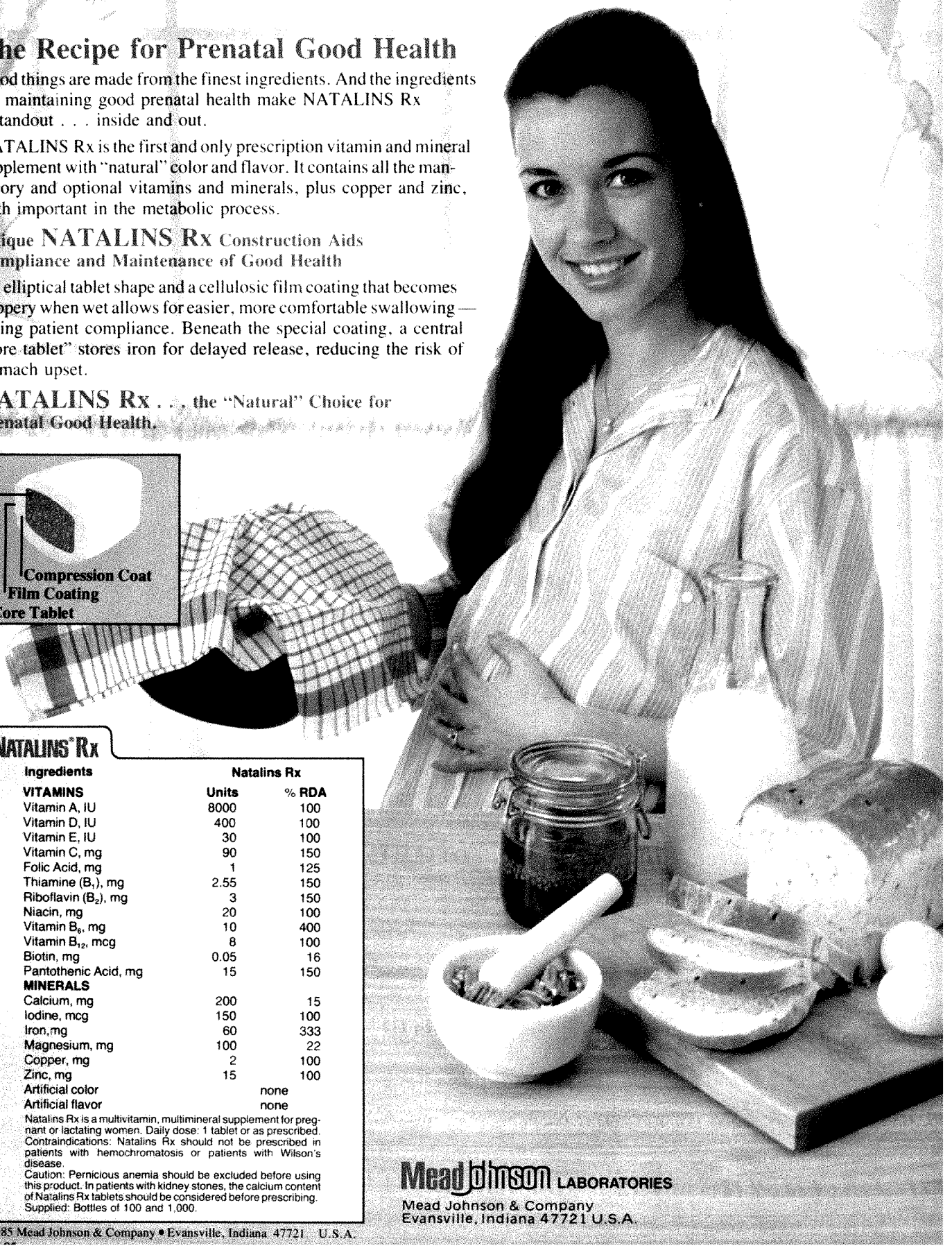
NATALINS Rx is the first and only prescription vitamin and mineral supplement with "natural" color and flavor. It contains all the mandatory and optional vitamins and minerals, plus copper and zinc, both important in the metabolic process.

Unique NATALINS Rx Construction Aids Compliance and Maintenance of Good Health

An elliptical tablet shape and a cellulosic film coating that becomes slippery when wet allows for easier, more comfortable swallowing — aiding patient compliance. Beneath the special coating, a central "core tablet" stores iron for delayed release, reducing the risk of stomach upset.

NATALINS Rx . . . the "Natural" Choice for Prenatal Good Health.





NATALINS® Rx

Ingredients	Natalins Rx	
VITAMINS	**Units**	**% RDA**
Vitamin A, IU	8000	100
Vitamin D, IU	400	100
Vitamin E, IU	30	100
Vitamin C, mg	90	150
Folic Acid, mg	1	125
Thiamine (B_1), mg	2.55	150
Riboflavin (B_2), mg	3	150
Niacin, mg	20	100
Vitamin B_6, mg	10	400
Vitamin B_{12}, mcg	8	100
Biotin, mg	0.05	16
Pantothenic Acid, mg	15	150
MINERALS		
Calcium, mg	200	15
Iodine, mcg	150	100
Iron, mg	60	333
Magnesium, mg	100	22
Copper, mg	2	100
Zinc, mg	15	100
Artificial color		none
Artificial flavor		none

Natalins Rx is a multivitamin, multimineral supplement for pregnant or lactating women. Daily dose: 1 tablet or as prescribed.
Contraindications: Natalins Rx should not be prescribed in patients with hemochromatosis or patients with Wilson's disease.
Caution: Pernicious anemia should be excluded before using this product. In patients with kidney stones, the calcium content of Natalins Rx tablets should be considered before prescribing.
Supplied: Bottles of 100 and 1,000.

Mead Johnson LABORATORIES
Mead Johnson & Company
Evansville, Indiana 47721 U.S.A.

©1985 Mead Johnson & Company • Evansville, Indiana 47721 U.S.A.

Contents continued from page 14A

Electromyographic observations on the human cervix during labor 691
Marjan Pajntar, M.D., Sc.D., Egidija Roškar, Sc.D., and Drago Rudel, M.Sc.
Kranj and Ljubljana, Yugoslavia

Significant bacteremia associated with replacement of intrauterine contraceptive device 698
Sarah Murray, B.M., J. B. Hickey, M.A., B.M., B.Ch., and Elizabeth Houang, M.B., Ch.B.
London, England

Measurement of antibody to *Mycoplasma hominis* by an enzyme-linked immunoassay and detection of class-specific antibody responses in women with postpartum fever 701
Mary B. Brown, Ph.D., Gail H. Cassell, Ph.D., William M. McCormack, M.D., and Jerry K. Davis, D.V.M., Ph.D.
Gainesville, Florida, Birmingham, Alabama, and Brooklyn, New York

Fetal biophysical profile scoring: Selective use of the nonstress test 709
F. A. Manning, M.D., I. Morrison, M.B., I. R. Lange, M.B., C. R. Harman, M.D., and P. F. C. Chamberlain, M.D.
Winnipeg, Manitoba, Canada

Embryonic trunk circumference: A new biometric parameter for estimation of gestational age 713
E. Albert Reece, M.D., Angela L. Scioscia, M.D., Jacqueline Green, M.S., R.D.M.S., Theresa Z. O'Connor, M.P.H., and John C. Hobbins, M.D.
New Haven, Connecticut

Levels of epidermal growth factor binding in third-trimester and term human placentas: Elevated binding in term placentas of male fetuses 716
Melinda J. Brown, Ph.D., Christine L. Cook, M.D., John L. Henry, M.S., and Gregory S. Schultz, Ph.D.
Louisville, Kentucky

Immunohistochemical localization of epidermal growth factor receptor and myc oncogene product in human placenta: Implication for trophoblast proliferation and differentiation 721
Takeshi Maruo, M.D., and Matsuto Mochizuki, M.D.
Kobe, Japan

Circannual rhythms in steroid receptor concentration in gynecologic and breast cancers 728
Joseph T. Chambers, Ph.D., M.D., Roberta Lawrence, M.P.H., Neil MacLusky, Ph.D., Arnold Eisenfield, M.D., and Peter E. Schwartz, M.D.
New Haven, Connecticut

Contents

An improved human tumor stem cell assay in ovarian cancer 730
Peter G. Rose, M.D., Antoinette Koolemans-Beynen, M.S., John G. Boutselis, M.D., John P. Minton, M.D., Ph.D., and George E. Milo, Ph.D.
Columbus, Ohio

Ontogeny of pituitary β-endorphin and related peptides in the human embryo and fetus 735
F. Facchinetti, M.D., A. R. Storchi, M.D., F. Petraglia, M.D., G. Garuti, M.D., and A. R. Genazzani, M.D.
Modena, Italy

BASIC SCIENCE SECTION

Effects of maternal anemia on cardiac output, systemic oxygen consumption, and regional blood flow in pregnant sheep 740
Daniel I. Edelstone, M.D., Mary E. Paulone, M.D., John J. Maljovec, M.D., and Margaret Hagberg, M.D.
Pittsburgh, Pennsylvania

Myocardial adenylate cyclase activity in ritodrine-treated pregnant rabbits 749
Christos G. Hatjis, M.D., and J. D. Bottoms, B.S.
Winston-Salem, North Carolina

Infusion of gonadotropin-releasing hormone agonist during pregnancy: Maternal and fetal responses in primates 755
V. M. Sopelak, Ph.D., and G. D. Hodgen, Ph.D.
Jackson, Mississippi, and Norfolk, Virginia

CORRESPONDENCE

Müllerіosis instead of endometriosis David B. Redwine, M.D. *Bend, Oregon* 761

Reply Joseph Sanfilippo, M.D. *Louisville, Kentucky* 761

Nurse-midwifery care and improved outcomes Barbara L. McFarlin, C.N.M., M.S., and Suzanne Trupin, M.D. *Champaign, Illinois* 761

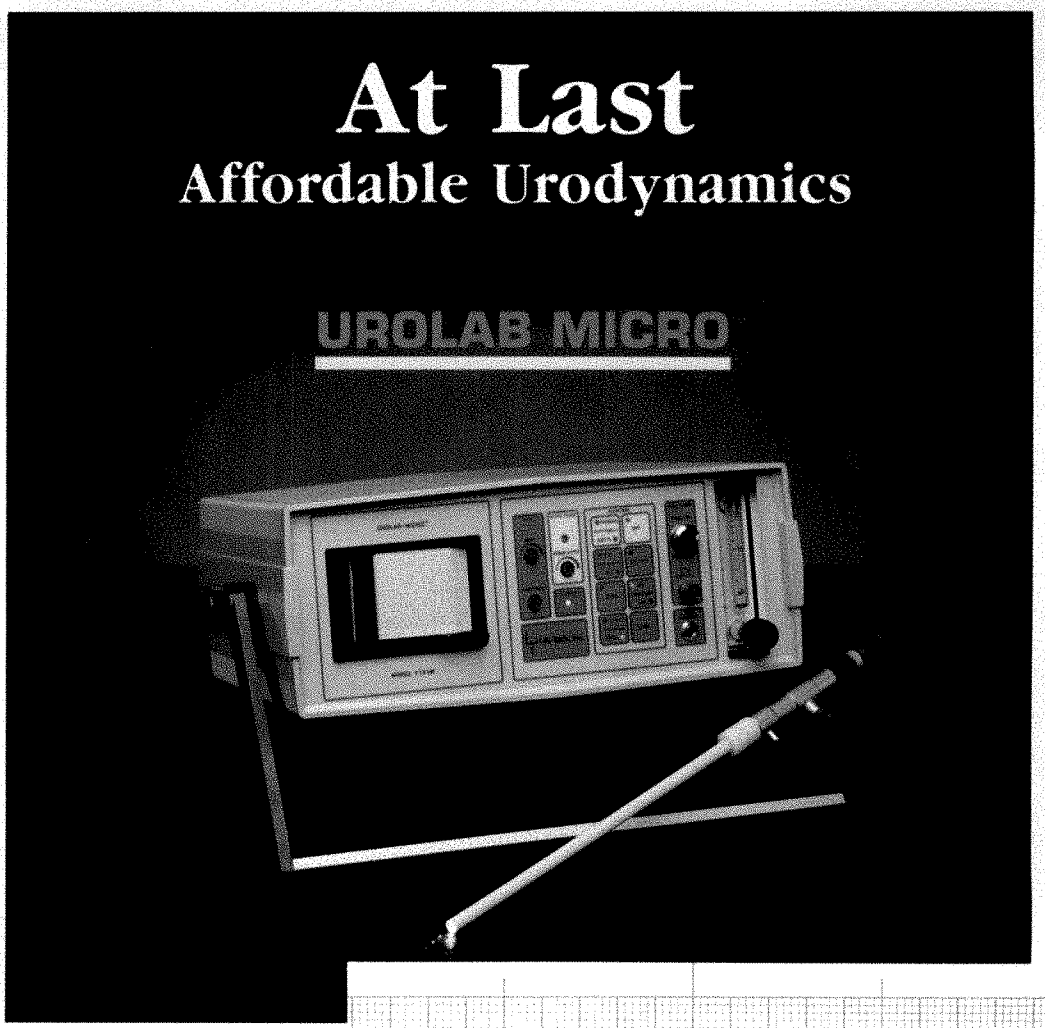
Reply Thomas R. Moore, M.D., William Origel, M.D., Thomas C. Key, M.D., and Robert Resnik, M.D. *San Diego, California* 762

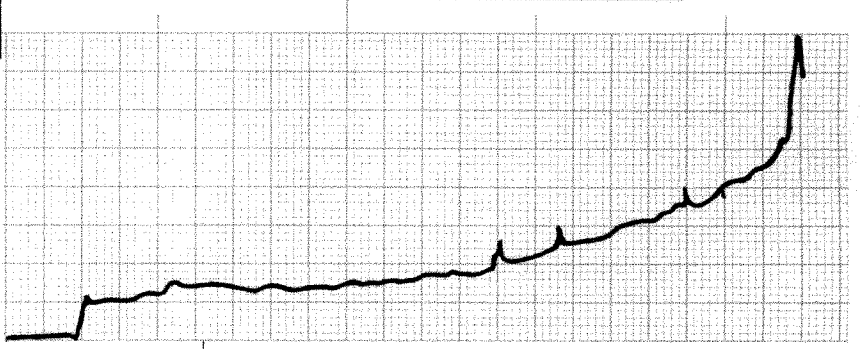
Sonographic sign for Down syndrome Francesco Giacomello, M.D. *Rome, Italy* 762

Reply Beryl Benacerraf, M.D. *Boston, Massachusetts* 763

(Contents continued on page 19A)

At Last
Affordable Urodynamics





Life-Tech®, Inc.
P.O. Box 36221, HOUSTON, TEXAS 77236-6221
1-800-231-9841, IN TEXAS CALL: (713) 495-9411
TELEX: 791364 LIFE TECH HOU

- Now economy in urodynamic equipment is available for gynecologists without sacrificing established Life-Tech quality.
- Urolab Micro for gas and water cystometry is only $2680, or lease it monthly for $99.69*.
- The system is fully expandable to include urethroscopy, cystoscopy, hysteroscopy, uroflowmetry and profilometry.
- Uroflowmetry, 0° and 70° endoscopes, and an internal light supply are available optionally.

For more information, call 1-800-231-9841
In Texas, call (713) 495-9411

LeaseCard

Lease your Life-Tech equipment with **LeaseCard**™. . . the ultimate in fast, convenient leasing.

Urolab Micro
Model 1151M for
$99.69 per month*
**Excluding tax*



Call Today

Exclusively from:
Trans Leasing International®
3000 Dundee Road, Northbrook, IL 60062
312/227-1000 (Illinois Res.)
TOLL FREE: 800-323-1180

©1984 by Trans Leasing International

LeaseCard is a Trademark of Trans Leasing International

Contents continued from page 17A

Abdominal pelvic pain syndrome: Fact or fiction R. W. Beard, P. W. Reginald, and S. Pearce *London, England* 763

Reply John C. Slocumb, M.D. *Albuquerque, New Mexico* 763

Use of midforceps and associated risk Emanuel A. Friedman, M.D., Sc.D., and David B. Acker, M.D. *Boston, Massachusetts* 764

Reply LeRoy J. Dierker, Jr., M.D., and Mortimer G. Rosen, M.D. *Cleveland, Ohio, and New York, New York* 765

Kell sensitization in pregnancy Fredric D. Frigoletto, Jr., M.D. *Boston, Massachusetts* 766

Reply Mark E. Caine, M.D., and Eberhard Mueller-Heubach, M.D. *Pittsburgh, Pennsylvania* 766

Selection of study patients with premature rupture of membranes Richard P. Porreco, M.D., and M. Shannon Burke, M.D. *Denver, Colorado* 766

Reply Walter J. Morales, M.D., Ph.D. *Tampa, Florida* 767

ITEMS

Items 768

Information for authors on pages 27A, 28A, and 29A

Index to advertisers on page 72A

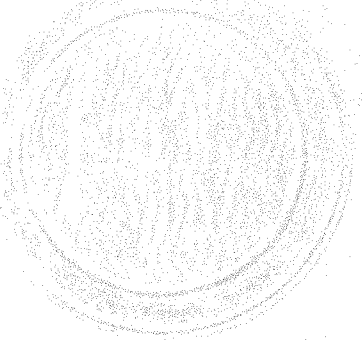
Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1987 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 156 (January-June) and Vol. 157 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

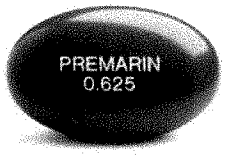
Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by The C. V. Mosby Company for libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that 10¢ per page is paid directly to CCC, 29 Congress St., Salem, MA 01970; phone (617)744-3350. The U.S. Government retains a nonexclusive, royalty-free license in and to any copyright on articles authored by government employees. 0002-9378/87 $00.00 + .10.

If your patient is estrogen deficient, she needs proven results a transdermal can't deliver...



She needs



PREMARIN®

(Conjugated Estrogens Tablets)

Only oral estrogen provides

- Proven protection against osteoporosis
- Decreased total cholesterol; increased HDL cholesterol; lowered LDL cholesterol
- Easy cycling with progestin
- Unsurpassed long-term safety

PREMARIN®

Complete estrogen therapy

Please see following page for brief summary of prescribing information.

The appearance of PREMARIN Tablets is a trademark of Ayerst Laboratories.

BRIEF SUMMARY *(FOR FULL PRESCRIBING INFORMATION AND PATIENT INFORMATION, SEE PACKAGE CIRCULAR.)*

PREMARIN® Brand of conjugated estrogens tablets, USP

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent, case-controlled studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than one year. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade. The three case-controlled studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semi-annual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.

The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing, in later life, a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1,000 exposures. Furthermore, a high percentage of such exposed women (from 30% to 90%) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb-reduction defects. One case-controlled study estimated a 4.7-fold increased risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than 1 per 1,000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. If PREMARIN is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the advisability of pregnancy continuation.

DESCRIPTION: PREMARIN (conjugated estrogens tablets, USP) for oral administration contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters. Tablets are available in 0.3 mg, 0.9 mg, 1.25 mg, and 2.5 mg strengths of conjugated estrogens.

INDICATIONS AND USAGE: Moderate to severe *vasomotor* symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression without associated vasomotor symptoms and they should not be used to treat such conditions.) Osteoporosis (abnormally low bone mass). Atrophic vaginitis. Kraurosis vulvae. Female castration.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

Concomitant Progestin Use: The lowest effective dose appropriate for the specific indication should be utilized. Studies of the addition of a progestin for 7 or more days of a cycle of estrogen administration have reported a lowered incidence of endometrial hyperplasia. Morphological and biochemical studies of the endometrium suggest that 10 to 13 days of progestin are needed to provide maximal maturation of the endometrium and to eliminate any hyperplastic changes. Whether this will provide protection from endometrial carcinoma has not been clearly established. There are possible additional risks which may be associated with the inclusion of progestin in estrogen replacement regimens. (See PRECAUTIONS.) The choice of progestin and dosage may be important; product labeling should be reviewed to minimize possible adverse effects.

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 2. Known or suspected estrogen-dependent neoplasia. 3. Known or suspected pregnancy (see Boxed Warning). 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

WARNINGS: Estrogens have been reported to increase the risk of endometrial carcinoma (see Boxed Warning). However, a recent large, case-controlled study indicated no increase in risk of breast cancer in postmenopausal women. A recent study has reported a 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease in women receiving postmenopausal estrogens.

Adverse effects of oral contraceptives may be expected at the larger doses of estrogen used to treat prostatic or breast cancer or postpartum breast engorgement; it has been shown that there is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. Users of oral contraceptives have an increased risk of diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis, thromboembolic disorders, or in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease. Large doses (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast have been shown to increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebitis. When doses of this size are used, any of the thromboembolic and thrombotic adverse effects should be considered a clear risk.

Benign hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has been reported in women taking estrogen-containing oral contraceptives. Increased blood pressure may occur with use of estrogens in the menopause and blood pressure should be monitored with estrogen use. A worsening of glucose tolerance has been observed in patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention, such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc. Prolonged administration of unopposed estrogen therapy has been reported to increase the risk of endometrial hyperplasia in some patients. Oral contraceptives appear to be associated with an increased incidence of mental depression. Patients with a history of depression should be carefully observed. Pre-existing uterine leiomyomata may increase in size during estrogen use. The pathologist should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, metabolic bone diseases associated with hypercalcemia, or in young patients in whom bone growth is not yet complete. If concomitant progestin therapy is used, potential risks may include adverse effects on carbohydrate and lipid metabolism.

The following changes may be expected with larger doses of estrogen:

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T_4 by column, or T_4 by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered.

d. Impaired glucose tolerance.

e. Decreased pregnanediol excretion.

f. Reduced response to metyrapone test.

g. Reduced serum folate concentration.

h. Increased serum triglyceride and phospholipid concentration.

As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

Long-term, continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. However, in a recent, large case-controlled study of postmenopausal women there was no increase in risk of breast cancer with use of conjugated estrogens.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy, including oral contraceptives: breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea; premenstrual-like syndrome; amenorrhea during and after treatment; increase in size of uterine fibromyomata; vaginal candidiasis, change in cervical erosion and in degree of cervical secretion; cystitis-like syndrome; tenderness, enlargement, secretion (of breasts); nausea, vomiting, abdominal cramps, bloating; cholestatic jaundice; chloasma or melasma which may persist when drug is discontinued; erythema multiforme; erythema nodosum; hemorrhagic eruption; loss of scalp hair; hirsutism; steepening of corneal curvature; intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea, and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION: 1. *Given cyclically for short-term use only.* For treatment of moderate-to-severe *vasomotor* symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause (0.3 mg to 1.25 mg or more daily).

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (eg, three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three- to six-month intervals.

2. *Given cyclically:* Osteoporosis. Female castration.

Osteoporosis —0.625 mg daily. Administration should be cyclic (eg, three weeks on and one week off).

Female castration—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Patients with an intact uterus should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

PREMARIN®

(Conjugated Estrogens Tablets)

Complete estrogen therapy

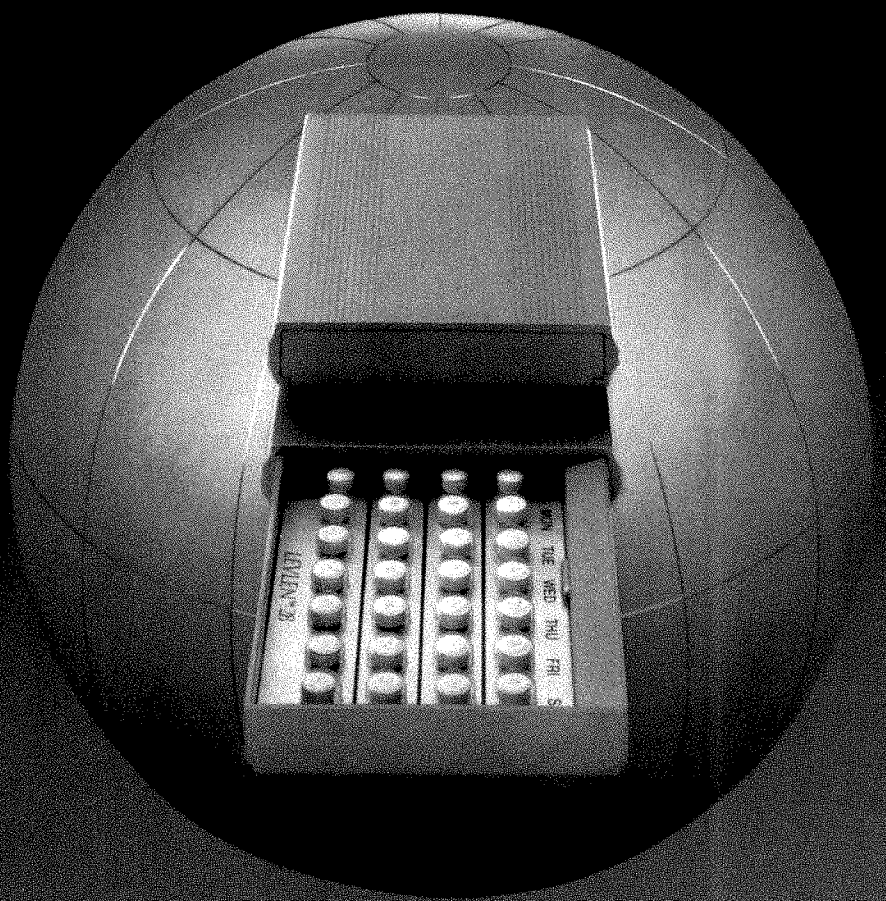
Ayerst® AYERST LABORATORIES
New York, NY 10017

6339/1286

LEVLEN™

(Levonorgestrel 0.15 mg and ethinyl estradiol 0.03 mg tablets)

THE MOST WIDELY PRESCRIBED OC FORMULA IN THE WORLD



The dose is low

There is no lower total hormonal dose in a monophasic OC.

Available in 28-day and 21-day Slidecase™ dispensers

The confidence is high

Incidence of breakthrough bleeding at cycle 6 is a low 5.6% based on 17 studies.[1]

Minimal impact on blood pressure[1] and lipids[2] in certain specific studies.*

*Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. The physician should remain alert to the earliest manifestations of any symptom of serious disease and discontinue oral contraceptive therapy when appropriate. These data represent only the results of selected studies, therefore, before prescribing, the physician should read and remain alert to all data found in the complete prescribing information for the product. Please also see brief summary of prescribing information on next page of advertisement.

LEVLEN™28 LEVLEN™21

BERLEX™ Dedicated to innovation in OB/GYN medicine

LEVLEN™ 28
LEVLEN™ 21

(Levonorgestrel 0.15 mg and ethinyl estradiol 0.03 mg tablets)

BRIEF SUMMARY

Each Levlen tablet contains 0.15 mg of levonorgestrel (d (-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxygon-4-en-3-one), a totally synthetic progestogen, and 0.03 mg of ethinyl estradiol (19-nor-17 α-pregna-1,3,5 (10)-trien-20-yne-3,17-diol) (7 pink inert tablets are included in Levlen 28).

Indications and Usage—Levlen is indicated for the prevention of pregnancy in women who elect to use OCs as a method of contraception.

Contraindications—OCs should not be used in women with any of the following conditions. 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during the use of OCs or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only OCs have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. (The rates given for Pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to six-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions:* There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.

4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs instead of other methods of contraception. Furthermore, a high percentage of such exposed women (. . .in 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only OCs are used), and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, an increased risk was apparent between 6 and 12 months of use.

7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. The clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.

9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.

10. *Bleeding Irregularities:* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in women who are not receiving OCs, since the drugs are more effective in preventing intrauterine than ectopic pregnancies.

12. *Breast-feeding:* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical examination being performed.

2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

Information for the Patient—See patient package labeling.

Laboratory Tests—1. The pathologist should be advised of OC therapy when relevant specimens are submitted.

2. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs:

a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thryoid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis, Mutagenesis, Impairment of Fertility—See "Warnings" section #3, 4 and 5 for information on carcinogenesis, mutagenesis and impairment of fertility.

Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."

Nursing Mothers—See "Warnings." Because of the potential for adverse reactions in nursing infants from OC tablets, a decision should be made whether to discontinue the drug, taking into account the importance of the drug to the mother.

Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis; pulmonary embolism; coronary thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertension; gallbladder disease; benign hepatomas; congenital anomalies.

There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally; gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted: premenstrual-like syndrome; cataracts; changes in libido; chorea; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; vaginitis; porphyria; hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—To achieve maximum contraceptive effectiveness, Levlen must be taken exactly as directed and at intervals not exceeding 24 hours.

Any time the patient misses 1 or 2 light-orange tablets, she should also use another method of contraception until she has taken a light-orange tablet daily for 7 consecutive days.

For full details on dosage and administration, see prescribing information in package insert.

References 1. Data submitted to FDA. **2.** Fotherby K. *Contraception* 1985;31:367-394.

BERLEX™
Dedicated to innovation in OB/GYN medicine

Berlex Laboratories, Inc., Wayne, New Jersey 07470
© 1986 Berlex Laboratories, Inc. All rights reserved. 431-12

American Journal of Obstetrics and Gynecology

Editors

Editors

JOHN I. BREWER, *Editor in Chief*
Sandra Fernandez, *Senior Editorial Assistant*

FREDERICK P. ZUSPAN, *Editor*
Meta M. Peterson, *Editorial Assistant*

E. J. QUILLIGAN, *Editor*
Rosalyn Vu, *Editorial Assistant*

ALBERT B. GERBIE, *Associate Editor*

Advisory committee on policy

William R. Anderson
Mason C. Andrews
E. Forrest Boyd
Amelia Cruz
Leo J. Dunn
A. Jerome Freeland
Morrie M. Gelfand
T. Terry Hayashi
Edgar O. Horger III
Hobart C. Hortman
Walter S. Keifer
Charles D. Kimball
Roger A. Lobo
Douglas J. Marchant
James H. Maxwell
Arthur M. McCausland
James A. Merrill
Frank C. Miller
Patrick T. Mohide
John C. Morrison
Richard Paul
Keith P. Russell
Ruth W. Schwartz
Joseph C. Scott, Jr.
Ruth Stockdale
Henry A. Thiede
Edward E. Wallach
Anne C. Wentz

Board of corresponding editors

Stuart Campbell, *England*
Luigi Carenza, *Italy*
Eliahu Sadovsky, *Israel*
Nils-Otto Sjöberg, *Sweden*
Masakuni Suzuki, *Japan*

Publisher
THE C. V. MOSBY COMPANY, 11830 Westline Industrial Drive, St. Louis, Missouri 63146
Kay G. Goehler, *Senior Manuscript Editor*

American Journal of Obstetrics and Gynecology

Societies

in addition to those listed on the front cover the Journal is the official publication of the following societies

NEW YORK OBSTETRICAL SOCIETY

OBSTETRICAL SOCIETY OF PHILADELPHIA

BROOKLYN GYNECOLOGICAL SOCIETY

ST. LOUIS GYNECOLOGICAL SOCIETY

NEW ORLEANS GYNECOLOGICAL AND OBSTETRICAL SOCIETY

THE OBSTETRICAL AND GYNECOLOGICAL SOCIETY OF MARYLAND

CHICAGO GYNECOLOGICAL SOCIETY

CINCINNATI OBSTETRICAL AND GYNECOLOGICAL SOCIETY

WASHINGTON GYNECOLOGICAL SOCIETY

PITTSBURGH OBSTETRICAL AND GYNECOLOGICAL SOCIETY

BOSTON OBSTETRICAL SOCIETY

LOUISVILLE OBSTETRICAL AND GYNECOLOGICAL SOCIETY

SEATTLE GYNECOLOGICAL SOCIETY

ALABAMA ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

AKRON OBSTETRICAL AND GYNECOLOGICAL SOCIETY

KANSAS CITY GYNECOLOGICAL SOCIETY

CENTRAL NEW YORK ASSOCIATION OF GYNECOLOGISTS AND OBSTETRICIANS

NEW JERSEY OBSTETRICAL AND GYNECOLOGICAL SOCIETY

IOWA OBSTETRIC AND GYNECOLOGIC SOCIETY

THE TEXAS ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

OKLAHOMA CITY OBSTETRICAL AND GYNECOLOGICAL SOCIETY

MEMPHIS OBSTETRICAL AND GYNECOLOGICAL SOCIETY

UTAH OBSTETRICAL AND GYNECOLOGICAL SOCIETY

ROCHESTER OBSTETRICAL AND GYNECOLOGICAL SOCIETY

ARKANSAS OBSTETRICAL AND GYNECOLOGICAL SOCIETY

TENNESSEE STATE OBSTETRICAL AND GYNECOLOGICAL SOCIETY

NEW YORK GYNECOLOGICAL SOCIETY

PACIFIC NORTHWEST OBSTETRICAL AND GYNECOLOGICAL ASSOCIATION

BUFFALO OBSTETRICAL AND GYNECOLOGICAL SOCIETY

SAN FRANCISCO GYNECOLOGICAL SOCIETY

JACKSON GYNECIC SOCIETY

INDIANA OBSTETRICAL AND GYNECOLOGICAL SOCIETY

THE MINNESOTA OBSTETRICAL AND GYNECOLOGICAL SOCIETY

Information for Authors

American Journal of Obstetrics and Gynecology

Copyright © 1987 by The C. V. Mosby Company

Editors

John I. Brewer, *Editor in Chief*
710 North Fairbanks Court, Chicago, IL 60611

Sandra Fernandez, *Senior Editorial Assistant*
Tel.: (312) 908-5266

Frederick P. Zuspan, *Editor*
The Ohio State University, Fifth Floor, Means Hall, 1654 Upham Dr., Columbus, OH 43210

Meta M. Peterson, *Editorial Assistant*
Tel.: (614) 293-8798

E. J. Quilligan, *Editor*
University of California, Davis, School of Medicine, Department of Obstetrics and Gynecology, 1621 Alhambra Blvd., Suite 2500, Sacramento, CA 95816

As of April 1, 1987
University of California, Irvine, California College of Medicine, Med Surge 1, Room 118, Irvine, CA 92717

Rosalyn Vu, *Editorial Assistant*
Tel.: (916) 453-5950

Albert B. Gerbie, *Associate Editor*
710 North Fairbanks Court, Chicago, IL 60611

Publisher
THE C. V. MOSBY COMPANY, 11830 Westline Industrial Drive, St. Louis, Missouri 63146

Kay G. Goehler, *Senior Manuscript Editor*
Tel.: (314) 872-8370

INFORMATION FOR AUTHORS

Editorial policies

The requirements for manuscripts submitted to the *American Journal of Obstetrics and Gynecology* conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors and published in *Annals of Internal Medicine* 1982;96(6):766-71. Certain requirements unique to our JOURNAL are provided in Information for Authors, published in each issue of the JOURNAL, and in more detail in the *Guide to Writing for the American Journal of Obstetrics and Gynecology*. The latter may be obtained from The C. V. Mosby Company or the Editors on request.

Manuscripts should be submitted to one of the three Editors as follows: (1) Dr. Brewer—all articles originating from the southeastern quadrant of the United States or Canada and those presented before one of the official sponsoring societies; (2) Dr. Zuspan—articles from the northeastern quadrant of the United States, Japan, Israel, Italy, and England, articles written for Clinical Opinion and Current Development, and Letters to the Editors; (3) Dr. Quilligan—articles from the north central states (including Ohio), states west of the Mississippi, Hawaii, Alaska, and abroad (except Japan, Israel, Italy, and England).

Most of the provisions of the Copyright Act of 1976 became effective on January 1, 1978. **Therefore, all manuscripts must be accompanied by the following written statement, signed by one author:** "The undersigned author transfers all copyright ownership of the manuscript [title of article] to The C. V. Mosby Company in the event the work is published. The undersigned author warrants that the article is original, is not under consideration by another journal, and has not been previously published. I sign for and accept responsibility for releasing this material on behalf of any and all co-authors." Authors will be consulted, when possible, regarding republication of material.

When submitting a manuscript, an author should include, if applicable, a full statement about all submissions and prior reports in any medium that might be regarded as prior or duplicate publication of the same or similar work. Copies of such material should be included with the submitted manuscript. Articles previously published in another language will not be considered.

It is assumed by the Editors that articles emanating from a particular institution are submitted with the approval of the requisite authority. Articles dealing with human experimentation that require local institutional approval must have this approval prior to submission of the article for publication and it should be so indicated in the Methods section. Reports of experiments on animals must indicate which guidelines for the care and use of the animals were followed.

Statements and opinions expressed in articles and communications herein are those of the author(s) and not necessarily those of the Editor(s) or Publisher and the Editor(s) and Publisher disclaim any responsibility or liability for such material. Neither Editor(s) nor Publisher guarantees, warrants, or endorses any product or service advertised in this publication, nor do they guarantee any claim made by the manufacturer of such product or service.

General requirements for preparation of manuscripts

The original and two good-quality copies of the manu-

script and three glossy prints of illustrations are required.

Manuscripts must be typed double spaced on one side only on 22 by 28 cm (8 1/2 by 11 inch) white bond paper with 1 inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, abstract, body of text, references, legends, and tables.

Title page. The title page should contain in sequence the title (concise and suitable for indexing purposes); author line with first name, middle initial, and last name of all authors and their highest academic degree (both M.D. and Ph.D. are acceptable); city(ies), state(s) in which the study was conducted; divisional, or departmental, and institutional affiliations at the time the study was performed; acknowledgement of source(s) of support; presented line, if applicable; disclaimers, if any; name and address of author to whom requests for reprints should be addressed (if reprints will not be available, it should be so stated); and name and address of author responsible for correspondence concerning the manuscript if different from author to whom reprint requests are addressed. At the bottom of the title page supply a short title for the running head not exceeding 52 characters (including word spaces).

Abstract page and key words/phrases. On manuscript page two is the abstract, also typed double spaced with the required margins and headed by the title of the article and author(s) name(s). Abstracts for regular articles, Current Investigation, Clinical Opinion, and Current Development may not exceed 150 words. Abstracts for case reports and brief communications may not exceed 50 words. Below the abstract list 3 to 5 *key words* or short phrases for indexing purposes.

Text. Do not hesitate to write your manuscript in the first-person, active voice if it is more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations since the emphasis is on the "action" rather than the person performing the action.

Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors*. Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify Material and Methods, Results, and Comment. Authors may wish to summarize their findings in a short paragraph at the end of the Comment section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods section* describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results section* present the detailed findings. Include mentions of all tables and/or figures. Avoid duplication of text and supporting material. Emphasize only your important observations; do not compare your observations to those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment section* state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgements. Acknowledge only persons who have made substantive contributions to the study.

References. Limit of 16 references, except for case reports and brief communications, limited to 2, and Current Development, for which there is no limit. Number references consecutively in the order in which they are mentioned in the text. Use the format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals." Journal titles should conform to abbreviations used in *Cumulated Index Medicus*.

Illustrations and tables. A reasonable number of halftone and line illustrations will be reproduced without charge, but special arrangements must be made with the Editors for color reproduction. For color photographs, submit original transparencies and two sets of unmounted prints on glossy paper with good color balance. For black and white illustrations, submit three sets of 3 by 4 inch (minimum) to 5 by 7 inch (maximum) unmounted glossy photographic prints. Figures must be cited consecutively in the text in Arabic numerals and identified thusly on the back of the print (gummed label with author(s) name(s), title of article, number, and top marked clearly). Do not use paper clips. Legends must accompany all figures; type double spaced on a single sheet of paper if possible and number with manuscript in sequence after the references. Titles should be included in the legend, *not* on the print.

All lettering must be done with commercially available paste-on letters (or numbers) or by a professional; typed or freehand lettering is not acceptable. All lettering must be in proportion to the drawing, graph, or photograph. Original drawings, appropriately done in black India ink, roentgenograms, and other material must be submitted as glossy photographic prints with good black and white contrast. Consistency in size within the article is strongly preferred. Any special instructions regarding sizing should be clearly noted. Illustrations will be returned only on the author's request.

Tables should be typed on separate sheets of paper and included at the end of the text, one to a page. They should be numbered in Roman numerals. Each table must be cited

Estimating length of manuscript

The length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A 4200 word text can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7 page article in the JOURNAL. The 2000 words of text alone make approximately 8 pages of manuscript typed double spaced with the required 1 inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 typed words in manuscript. Thus if a greater number of illustrations and tables are used, the length of the text should be adjusted accordingly.

in sequence at an appropriate point in the text. Captions should be brief yet indicate clearly the purpose or content of each table and each column precisely defined by headings. Abbreviations and special designations should be explained in a footnote to the table. Special arrangements must be made with the Editors for elaborate tables because of space limitations.

Illustrations and tables should supplement, not duplicate, the text; presentation in either one or the other will suffice.

Permissions and photographic consent. Previously published tables and figures must be correctly referenced and written permission from the copyright holder included with the manuscript. Photographs of identifiable persons must be accompanied by signed releases or else all recognizable features masked.

Requirements for special sections

Case reports and brief clinical and basic science communications. Limit of 700 words, 2 references. Include abstract of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures are used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

Current Investigation. Same requirements as for a regular article.

Clinical Opinion. Limit of 3000 words, 16 references. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Current Development. Limit of 6000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Correspondence. Two types of correspondence will be considered for publication. (1) A Letter to the Editors commenting on an article that has appeared in the JOURNAL should be brief and directly related to the published article. The editorial staff reserves the right to shorten letters if necessary and to make minor editorial alterations without reference to the writer. Letters may be published together with a reply from the original author. As space for letters is limited, only a selection of letters submitted may be published. (2) A brief case presentation or a short report of a pertinent observation in the form of a Letter to the Editors will be considered for publication. All letters should be typed double spaced. Letters should be sent to Dr. Zuspan.

Announcements. Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

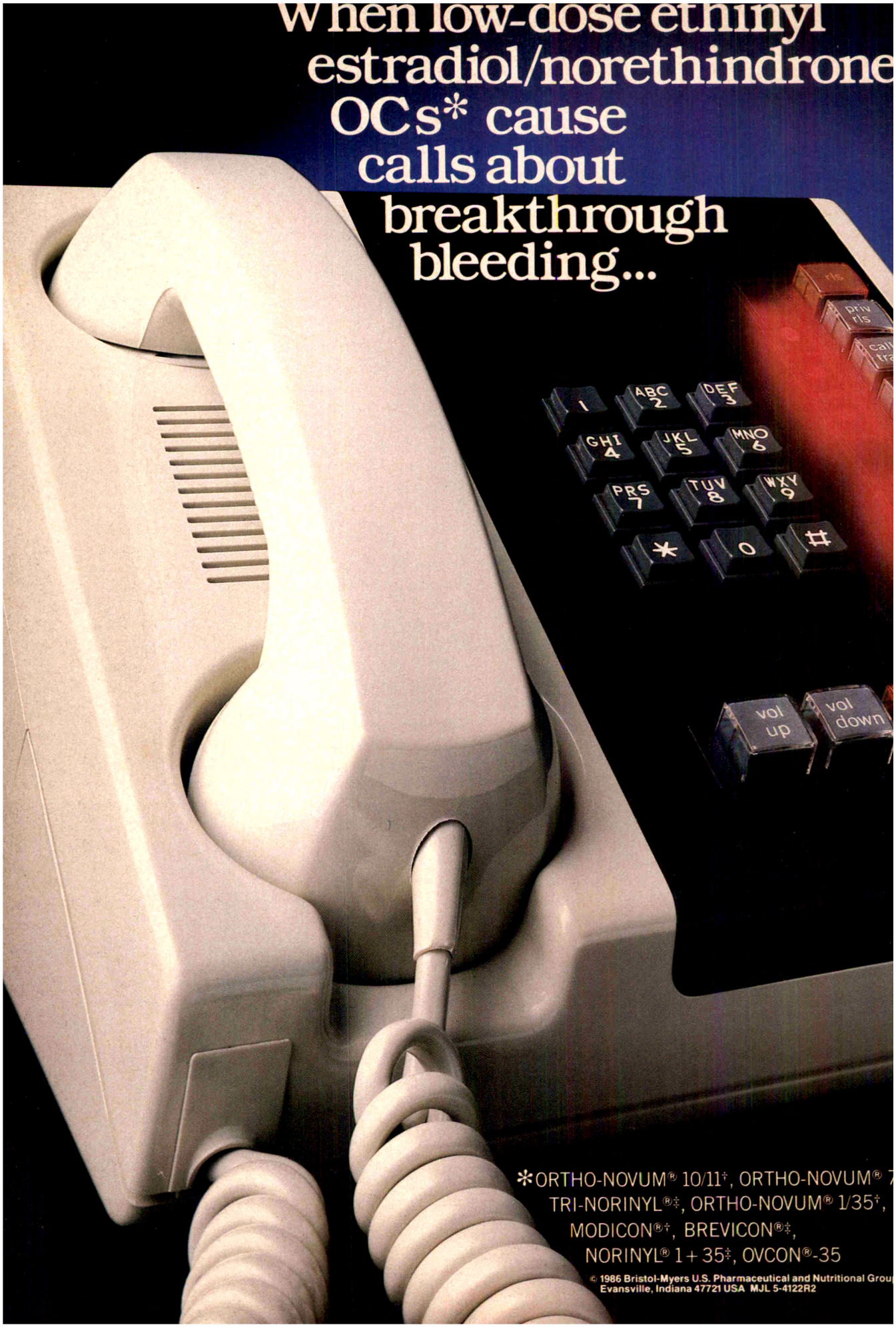
Books. Books received will be listed in the JOURNAL. They should be sent to Dr. Gerbie. No books will be returned.

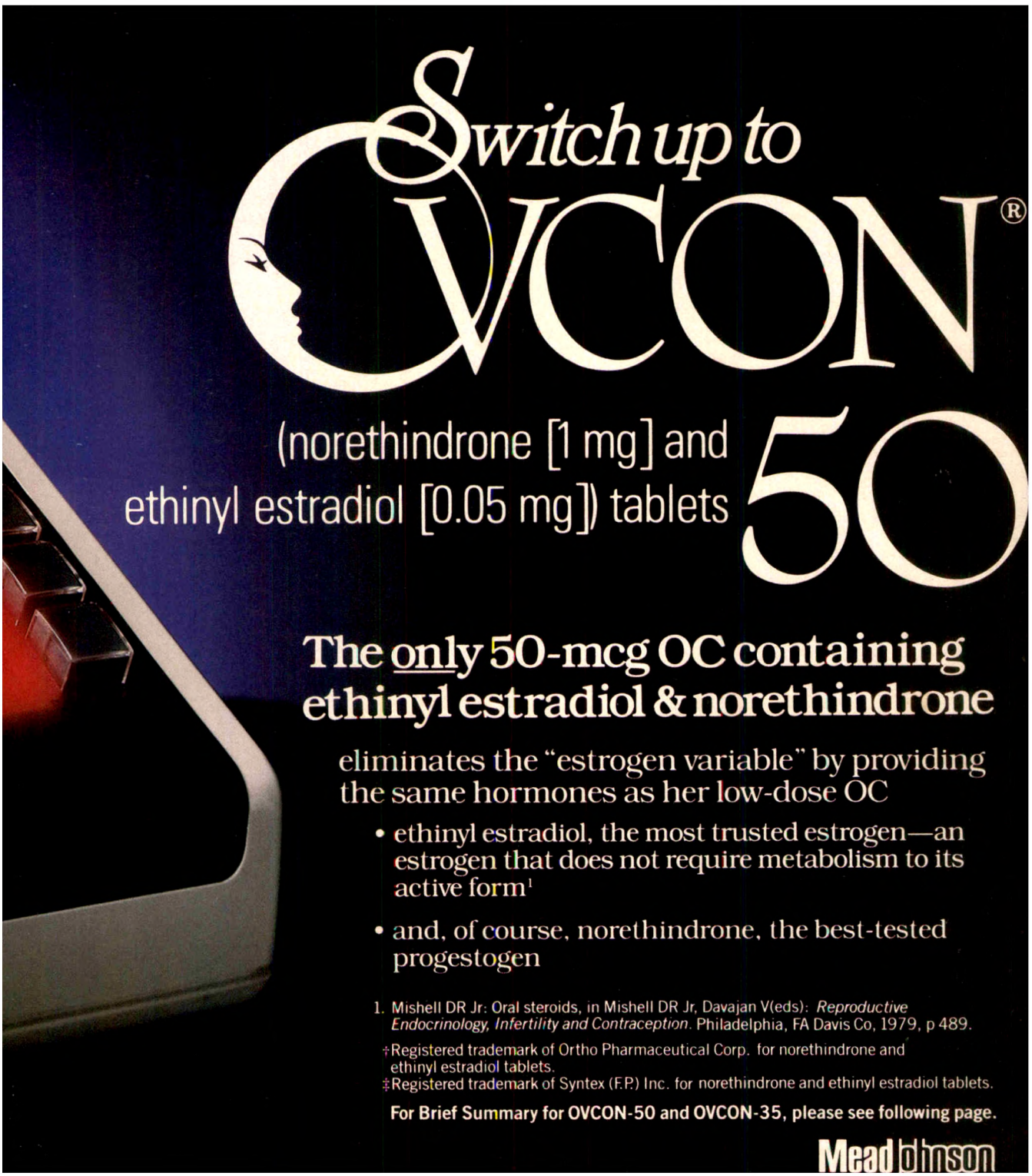
Reprints. Reprints of articles must be obtained from the author. The corresponding author will receive a price schedule and order form at the time of publication. Reprints in quantities must be ordered from the Publisher with the author's consent.

Business communications. Communications of a business nature and all advertising communications should be addressed to: Journal Publisher, The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, or call Journal Advertising Production Manager (314) 872-8370.

Checklist

—Letter of submission
—Copyright transfer letter
—Original and two xerographic copies of manuscript
—Title page
—Title of article
—Full name(s) and affiliations of author(s)
—Author to whom correspondence is to be sent
—Reprint request line or line stating reprints not available
—Short title
—Abstract (double spaced), 3 to 5 key words/phrases
—Article proper (double spaced)
—References (double spaced), on a separate sheet
—Legends (double spaced), on a separate sheet
—Tables (double spaced), each on a separate sheet
—Illustrations, properly labeled (three copies of glossy prints)
—Permission to reproduce published material


When low-dose ethinyl estradiol/norethindrone OCs* cause calls about breakthrough bleeding...
vol up
vol down
*ORTHO-NOVUM® 10/11†, ORTHO-NOVUM® 7
TRI-NORINYL®‡, ORTHO-NOVUM® 1/35†,
MODICON®†, BREVICON®‡,
NORINYL® 1+35‡, OVCON®-35
© 1986 Bristol-Myers U.S. Pharmaceutical and Nutritional Grou
Evansville, Indiana 47721 USA MJL 5-4122R2


Switch up to
OVCON®
(norethindrone [1 mg] and
ethinyl estradiol [0.05 mg]) tablets
50
The only 50-mcg OC containing
ethinyl estradiol & norethindrone
eliminates the "estrogen variable" by providing
the same hormones as her low-dose OC
• ethinyl estradiol, the most trusted estrogen—an estrogen that does not require metabolism to its active form[1]
• and, of course, norethindrone, the best-tested progestogen
1. Mishell DR Jr: Oral steroids, in Mishell DR Jr, Davajan V(eds): *Reproductive Endocrinology, Infertility and Contraception*. Philadelphia, FA Davis Co, 1979, p 489.
†Registered trademark of Ortho Pharmaceutical Corp. for norethindrone and ethinyl estradiol tablets.
‡Registered trademark of Syntex (F.P.) Inc. for norethindrone and ethinyl estradiol tablets.
For Brief Summary for OVCON-50 and OVCON-35, please see following page.
Mead Johnson

OVCON®-50

(norethindrone [1 mg] and ethinyl estradiol [0.05 mg]) tablets

OVCON®-35

(norethindrone [0.4 mg] and ethinyl estradiol [0.035 mg]) tablets

Indications and Usage: Ovcon is indicated for the prevention of pregnancy. Some combination products containing 35 mcg. or less of ethinyl estradiol report slightly higher pregnancy rates than are reported for the higher dose combination products.

Dose-related Risk of Thromboembolism from Oral Contraceptives: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing 50 mcg. or less of estrogen.

Contraindications: Oral contraceptives should not be used in women with any of the following conditions: 1) Thrombophlebitis or thromboembolic disorders. 2) A past history of deep vein thrombophlebitis or thromboembolic disorders. 3) Cerebral vascular or coronary artery disease. 4) Known or suspected carcinoma of the breast. 5) Known or suspected estrogen dependent neoplasia. 6) Undiagnosed abnormal genital bleeding. 7) Known or suspected pregnancy. 8) Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

Warnings:

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. Women who use oral contraceptives should be strongly advised not to smoke. Use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1) *Thromboembolic Disorders and Other Vascular Problems.* An increased risk of thromboembolic and thrombotic disease associated with use of oral contraceptives is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. In a study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater. An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported. Studies conducted in the United Kingdom found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction compared to nonusers who do not smoke. Oral contraceptive users who smoke have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. The amount of smoking is a very important factor. British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives; preparations containing 100 mcg. or more of estrogen were associated with a higher risk of thromboembolism than those containing 50–80 mcg. of estrogen. The relative risk of thromboembolic disease associated with progestin-only oral contraceptives has not been determined. Cases of thromboembolic disease have been reported in women using progestin-only products, and they should not be presumed to be risk-free. The overall excess mortality rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15–34, 5/100,000; ages 35–44, 33/100,000; ages 45–49, 140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. The highest risk was found in heavy cigarette smokers (15 or more cigarettes per day) who used oral contraceptives and were aged 40 or older. Women who smoke should be advised not to use oral contraceptives. The use of oral contraceptives in women over age 40 with other risk factors is not recommended. The mortality associated with all of the methods of birth control is low compared to the risk of childbirth, with the exception of oral contraceptive users who smoke and are over age 40. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and for myocardial infarction is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia, and especially by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. If feasible, oral contraceptives should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2) *Ocular Lesions.* There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue the medication if there is unexplained sudden or gradual, partial or complete loss of vision; sudden onset of proptosis or diplopia; papilledema; or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3) *Carcinoma.* Long term administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. In humans, one study investigated the first 21 cases of endometrial adenocarcinoma in women on oral contraceptives reported to a registry. Of those women without predisposing risk factors for this disease, nearly all occurred in women who had used a sequential oral contraceptive. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestin-only oral contraceptives. No increase in breast cancer in women taking oral contraceptives has been reported although one study reported an increased risk of breast cancer in subgroups of women treated using oral contraceptives with documented benign breast disease. There is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives. 4) *Hepatic Adenoma.* Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study reported a higher risk associated with oral contraceptive formulations with high hormonal potency. Although benign and rare, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short term as well as long term users of oral contraceptives. Two studies relate the risk with duration of contraceptive use, the risk being much greater after 4 or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5) *Use in Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring.* Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. A high percentage of such exposed women (30% to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Similar data are not available with the use of other estrogens but it cannot be presumed that they would not induce similar changes. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000 live births. In the past, female abortion. There is considerable evidence that estrogens are ineffective for these indicatio there is no evidence from well-controlled studies that progestins are effective for these Increases in chromosomal aberrations have been reported in women who become pregnan after ceasing oral contraceptive therapy. Embryos with these anomalies are virtually spontaneously aborted. Whether there is an overall increase in spontaneous abortion of pregn conceived soon after stopping oral contraceptives is unknown. It is recommended that for any who has missed two consecutive periods, pregnancy should be ruled out before continui contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possib pregnancy should be considered at the time of the first missed period and further use contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirm patient should be apprised of the potential risks to the fetus and the advisability of preg continuation should be discussed in light of these risks. It is also recommended that wome discontinue oral contraceptives with the intent of becoming pregnant use an alternate fo contraception for a period of time before attempting to conceive. Many clinicians recommen months. Administration of progestin-only or progestin-estrogen combinations to induce with bleeding should not be used as a test for pregnancy. 6) *Gall Bladder Disease.* Studies have repo increased risk of surgically confirmed gallbladder disease appearing after one year c contraceptive use and doubling of the risk after 4 or 5 years of use. 7) *Carbohydrate and Metabolic Effects.* A decrease in glucose tolerance has been observed in a significant percent patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be ca observed while receiving oral contraceptives. Increased serum levels of triglycerides an phospholipids have been observed in oral contraceptive users. The clinical significance observation is unknown at this time. 8) *Elevated Blood Pressure.* An increase in blood pressu been reported in women receiving oral contraceptives. The prevalence of hypertension contraceptive users may be no higher than nonusers in the first year of oral contraceptive u increases with longer exposure and in the fifth year of use is two and one-half to three tim reported prevalence in the first year. Women who previously had hypertension during pregnanc be more likely to develop elevation of blood pressure when given oral contraceptives. 9) *Head* The onset or exacerbation of migraine or development of headache of a new pattern wh recurrent, persistent or severe, requires discontinuation of oral contraceptives and investiga the cause. 10) *Bleeding Irregularities.* Breakthrough bleeding, spotting and amenorrhea are fre reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all ca irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiag persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measur indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or cha another formulation may solve the problem. Changing to an oral contraceptive with a higher est content, while potentially useful in minimizing menstrual irregularity, should be done necessary since this may increase the risk of thromboembolic disease. Women with a past hist oligomenorrhea or secondary amenorrhea or young women without regular cycles may h tendency to remain anovulatory or to become amenorrheic after discontinuation o contraceptives. Women with these preexisting problems should be advised of this possibili encouraged to use other contraceptive methods. 11) *Ectopic Pregnancy.* Ectopic as w intrauterine pregnancy may occur in contraceptive failures. However, in oral contraceptive fai the ratio of ectopic to intrauterine pregnancies is higher than in women not using oral contrace since the drugs are more effective in preventing intrauterine rather than ectopic pregnanc higher ectopic-intrauterine ratio has been reported with both combination products and prog only oral contraceptives. 12) *Breast Feeding.* A small fraction of the hormonal agents i contraceptives has been identified in the milk of mothers receiving these drugs. The long- effect to the nursing infant cannot be determined at this time.

Precautions: 1) A complete medical and family history should be taken prior to the initiation contraceptives. Examination should include special reference to blood pressure, breasts, abd and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a genera oral contraceptives should not be prescribed for longer than 1 year without another ph examination being performed. 2) Under the influence of estrogen-progestogen prepara preexisting uterine leiomyomata may increase in size. 3) Patients with a history of ps depression should be carefully observed and the drug discontinued if depression recurs to a s degree. 4) Because oral contraceptives may cause some degree of fluid retention, conditions might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, carc renal insufficiency or asthma require careful observation. 5) Patients with a past history of jau during pregnancy have an increased risk of recurrence of jaundice while receiving oral contrac therapy. If jaundice develops in any patient receiving such drugs, the medication shou discontinued. 6) Steroid hormones may be poorly metabolized in patients with impaired function and should be administered with caution in such patients. 7) Oral contraceptive user have disturbances in normal tryptophan metabolism which may result in a relative pyrid deficiency. The clinical significance of this is yet to be determined. 8) Serum folate levels m depressed by oral contraceptive therapy. This may complicate subsequent pregnancy with reg folate deficiency. 9) The pathologist should be advised of oral contraceptive therapy when rel specimens are submitted. 10) Certain endocrine and liver function tests and blood component be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein rete b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; incr norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) le to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), column, or T_4 by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the ele TBG; free T_4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced resp to metyrapone test. 11) The active yellow tablets and the inert green tablets in Ovcon-50 (21 a day regimens) and the inert green tablets in the 28 day regimen of Ovcon-35 contain FD&C Yello 5 (tartrazine) which may cause allergic-type reactions (including bronchial asthma) in c susceptible individuals. Although the overall incidence of FD&C Yellow No. 5 (tartrazine) sens in the general population is low, it is frequently seen in patients who also have a hypersensitivity.

Information for the Patient: Detailed Patient Labeling has been prepared for use by the patient an been made available for distribution by the pharmacist.

Drug Interactions: Reduced efficacy and increased incidence of breakthrough bleeding have associated with concomitant use of rifampin. A similar association has been suggested barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Adverse Reactions: An increased risk of the following serious adverse reactions has been assoc with the use of oral contraceptives (see **Warnings**): Thrombophlebitis, pulmonary embo coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gall bl disease, congenital anomalies. There is evidence of an association between the following condi and the use of oral contraceptives, although additional confirmatory studies are needed: Mese thrombosis; benign hepatomas; neuro-ocular lesions, e.g., retinal thrombosis and optic neu The following adverse reactions have been reported in patients receiving oral contraceptives an believed to be drug related: Nausea and/or vomiting, usually the most common adverse react occur in approximately 10% or less of patients during the first cycle (other reactions, as a ge rule, are seen much less frequently or only occasionally); gastrointestinal symptoms (su abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuar treatment; edema; chloasma or melasma which may persist; breast changes (tender enlargement, and secretion); change in weight (increase or decrease); change in cervical erosio cervical secretion; possible diminution in lactation when given immediately postpartum; chole jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depres reduced tolerance to carbohydrates; vaginal candidiasis; changes in corneal curvature (steeper intolerance to contact lenses. The following adverse reactions have been reported in users o contraceptives, and the association has been neither confirmed nor refuted: Premenstrua syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndr headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, eryt nodosum, hemorrhagic eruption, vaginitis, porphyria.

Acute Overdosage: Serious ill effects have not been reported following acute ingestion of large c of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal blee may occur in females.

Mead Johnson LABORATORIES

C-V21
MJL 5-41

American Journal of Obstetrics and Gynecology

Founded in 1920

volume 156 *number* 3 MARCH 1987

EDITORIAL

Summary of analysis of published articles

September 1, 1985, through August 31, 1986

Each year a retrospective analysis of the articles published in the JOURNAL is presented to the readers and also to the Editorial Board and the Advisory Committee on Policy. Over the past seventeen years we have compiled data on the classification, category, and type of articles accepted and published. A comparison of the data for the last two years is presented in Table I. A comparison of publication time in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY of regular articles (excluding society articles) for 1981-1982 through 1985-1986 is shown graphically in Fig. 1, on page 526. Readers and authors should note the time from acceptance to publication was 3.64 months for the period analyzed in 1986.* The circle graphs on page 526 show the articles and page utilization for 1986 by classification (Fig. 2A) and by category (Fig. 2B).

*Publication time of regular articles from time of acceptance to publication: 1982, 4.72 months; 1983, 4.95 months; 1984, 5.60 months; 1985, 4.90 months; 1986, 3.64 months.

Table I. Comparison of data on classification, category, and type of articles published in 1985 and 1986

	1985			*1986*		
	No. of articles	*Pages*	*Average length per article*	*No. of articles*	*Pages*	*Average length per article*
Classification of article:						
Clinical	273	1175		363	1478	
Clinical Investigation	206	1059		117	631	
Basic Research	115	619		88	512	
Total	**594**	**2853**		**568**	**2621**	
Category of articles:						
Obstetrics	230	1080		216	872	
Gynecology	188	948		172	896	
Fetus, Placenta, Newborn	175	819		167	793	
Education	1	6		13	60	
Total	**594**	**2853**		**568**	**2621**	
Composition of JOURNAL based on type of article:						
Regular	317	1653	5.2	286	1423	5.0
Society	145	862	6.0	140	825	5.9
Brief Communication/Case Report	103	169	1.6	115	193	1.7
Current Development	6	64	10.7	8	89	11.1
Current Investigation	8	25	3.1	2	9	4.5
Clinical Opinion	15	80	5.3	17	82	4.8
Total	**594**	**2853**		**568**	**2621**	

Continued

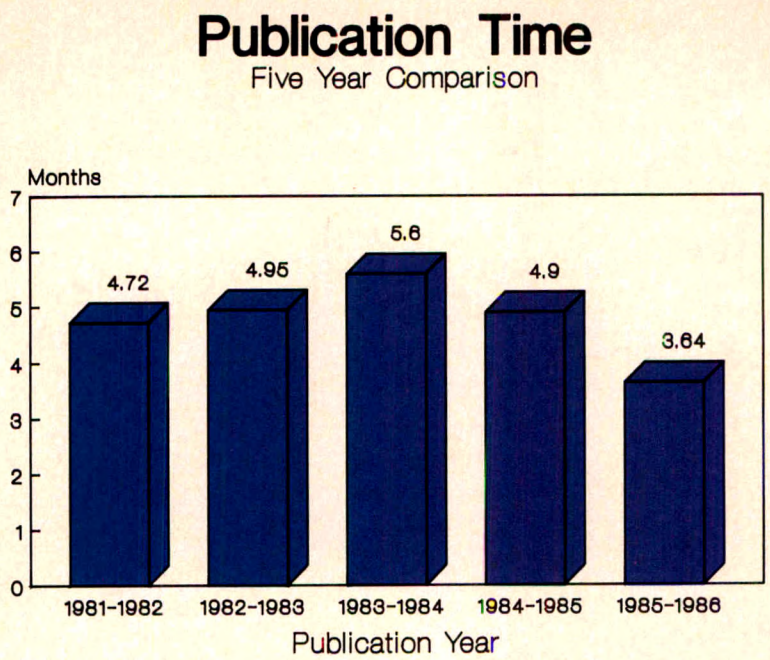


Fig. 1. Five-year comparison of publication time of regular articles (excluding society articles) in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY.

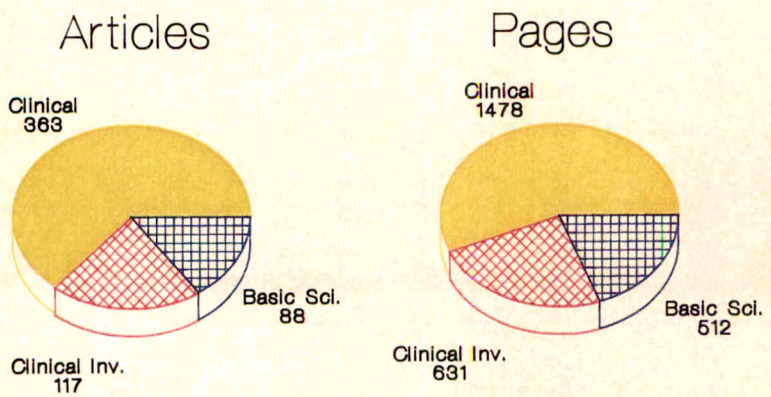


Fig. 2A. Articles and page utilization for 1986 by classification.

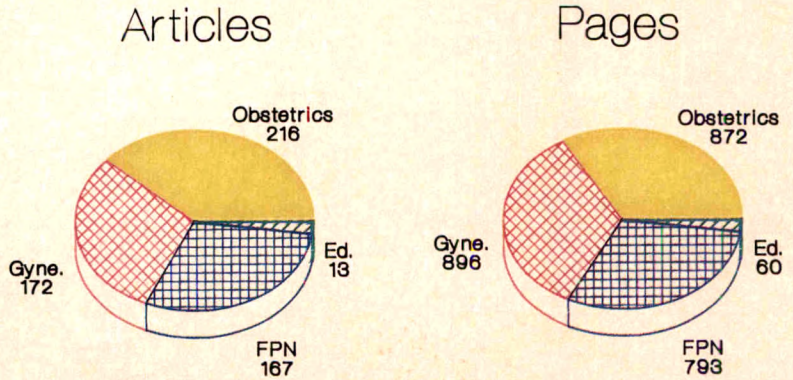


Fig. 2B. Articles and page utilization for 1986 by category.

Clinical Opinion

The use and misuse of the fetal biophysical profile

Anthony M. Vintzileos, M.D., Winston A. Campbell, M.D., David J. Nochimson, M.D., and Paul J. Weinbaum, M.D.

Farmington, Connecticut

The value of the fetal biophysical profile in determining fetal well-being has been well documented. The increasing clinical use of the fetal biophysical profile, however, has led to the recognition of frequent errors in the interpretation and application of this modality. These errors may result in unnecessary interventions or adverse perinatal outcome. The most frequent errors in the interpretation and application of the fetal biophysical profile are described; in an attempt to minimize the misuse of the fetal biophysical profile, an alternative protocol of antepartum fetal evaluation is suggested based upon the information obtained from the biophysical monitoring of the fetus. (AM J OBSTET GYNECOL 1987;156:527-33.)

Key words: Fetal biophysical profile, antepartum fetal evaluation

The fetal biophysical profile (nonstress test, fetal movements, fetal breathing movements, fetal tone, amniotic fluid volume, placental grading) has been shown to be more accurate in the identification of the compromised fetus than any other single test during the antepartum period.[1-10] Its predictive value in antepartum fetal surveillance has been very well documented for patients with intact as well as ruptured membranes.[1-10] In high-risk patients with intact membranes it has been shown to be more accurate than the contraction stress test[5] and the nonstress test.[11] The method used for determination of the fetal biophysical profile has been previously reported.[1, 5] Fetal biophysical variables have been assigned an arbitrary score of 2 when normal and 0 when abnormal.[1-3] In our institution the fetal biophysical variables, including placental grading, have been assigned an arbitrary score of 2, 1, or 0 (modified from the biophysical scoring of Manning et al.[1-3]). Regardless of the differences in the scoring criteria, in all reported studies a fetal biophysical profile score of 8 or more has been considered reassuring of fetal well-being and a score of 7 or less nonreassuring.[1-10] In the past, management protocols were suggested based on the biophysical profile scoring.[1-3, 5, 6]

The purpose of this communication is to discuss the physiology and pathophysiology of the fetal biophysical activities so that the clinician understands what the presence or absence of a given biophysical activity means, to describe the most frequent errors in the interpretation and clinical application of the fetal biophysical profile, and to present a simple approach of antepartum determination of fetal well-being using the information obtained from the biophysical monitoring of the fetus.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Connecticut Health Center.
Reprint requests: Anthony M. Vintzileos, M.D., Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Connecticut Health Center.

Physiology and pathophysiology of fetal biophysical activities

Dynamic biophysical activities are not random events but rather are initiated and regulated by complex integrated mechanisms of the fetal central nervous system. The presence of a normal biophysical activity is indirect evidence that a given portion of the central nervous system that controls the activity is intact and functioning and therefore nonhypoxemic. The absence of a given fetal biophysical activity, however, is much more difficult to interpret, since it may reflect either pathologic depression or normal periodicity. The periodicity in the biophysical activities has been shown[12, 13] to be short term (20 to 40 minutes) or long term, similar to circadian (diurnal) rhythms seen in extrauterine life.

Factors that cause fetal central nervous system depression may reduce or abolish fetal biophysical activities. Fetal hypoxemia produces a dramatic decrease of fetal heart rate reactivity and fetal breathing movements and, when severe, will result in reduced or absent fetal movements and fetal tone.[5] Central nervous sys-

tem depressants such as analgesics (morphine, meperidine), sedatives (barbiturates, diazepam), and anesthetics (halothane) reduce or inhibit fetal biophysical activities. In contrast, central nervous system stimulants such as hyperglycemia and catecholamines may result in increased biophysical activities.

The nervous impulses that initiate fetal biophysical activities arise from different anatomic sites within the brain.[5, 7, 14] The sensitivity of these areas to hypoxemia is unknown, but it has been strongly suggested that variations in sensitivity exist.[5, 7, 14] During the fetal neurodevelopment, a higher oxygen level is required for newly developing central nervous system centers and for reflex biophysical activities. The biophysical activities that become active first in fetal development are the last to disappear when asphyxia arrests all biophysical activities.[14] For example, the fetal tone center (cortex-subcortical area) is the earliest to function during intrauterine life (7.5 to 8.5 weeks)[14] and is the last to disappear during asphyxia. The fetal movement center (cortex-nuclei) starts functioning at approximately 9 weeks,[15] and accordingly, it is more sensitive to hypoxia than the fetal tone center. Although isolated fetal breathing movements have been documented as early as 13 to 14 weeks, diaphragmatic contraction and regular fetal breathing do not occur until 20 to 21 weeks,[15] when the fetal breathing movement center (ventral surface of the fourth ventricle) is fully developed and functioning. The fetal heart rate reactivity center (posterior hypothalamus, medulla), which starts operating by the end of the second trimester or early third trimester, is therefore the most sensitive to hypoxia. In the antepartum fetal evaluation the presence or absence of the acute markers of the fetal condition (nonstress test, fetal breathing movements, fetal movements, and fetal tone) will determine the level of fetal compromise at the time of testing.[5] The fetus with suboptimal oxygenation will usually present with nonreactive nonstress testing and absence of fetal breathing at the initial stages of hypoxia; if hypoxia becomes worse, the fetal body movements and fetal tone will be abolished (gradual hypoxia concept). In our reported experience[5, 7] all hypoxic fetuses—as determined by umbilical cord pH measurements—had nonreactive nonstress tests and in no cases was fetal breathing present. These hypoxic fetuses had perinatal death rate of 0 when fetal movements were normal. When the fetal movements were decreased or absent, the perinatal death rate ranged from 20% to 75%. When the fetal tone also was poor, the perinatal mortality was 100%. This correlation between the gradual hypoxia concept and perinatal otucome has been further confirmed by our subsequent experience with a total of 6543 fetal biophysical profiles.

Amniotic fluid volume, unlike the other biophysical variables, is not acutely influenced by alterations in fetal central nervous system function. It is included in the fetal biophysical profile because of the high correlation between decreased amniotic fluid volume and increased incidence of abnormal pregnancy outcome.[3, 5, 16] It is a chronic marker of the fetal condition. The mechanism by which chronic fetal asphyxia results in oligohydramnios is not fully elucidated but may involve hypoxia-induced reflex redistribution of cardiac output with preferential shunting of blood away from the lung and kidney, the two major sources of fluid production in later pregnancy.[17] Fetuses with decreased amniotic fluid volume are at high risk for cord compression and in utero death.[3, 5, 7, 16] In our institution placental grading is also included in the fetal biophysical profile, since cases with grade III placentas have been found to be associated with increased incidence of abnormal fetal heart rate patterns and abruptio placentae during labor.[5, 7] The value of including placental grading is to alert the physician during the intrapartum period in the presence of a grade III placenta.

Misuse of the fetal biophysical profile

The most common errors that clinicians make when they use the fetal biophysical profile as a means of antepartum fetal evaluation are outlined below:

Management decisions based on the score alone. This is the most frequent error among clinicians who use the fetal biophysical profile for fetal surveillance. This error may lead to unnecessary interventions or preventable adverse perinatal outcome. Although it has been repeatedly stated[3, 5, 7] that management should be based not solely on the score but also on the score components, this principle has not been emphasized strongly enough in the literature; the end result is that clinicians tend to base their decisions on the score alone. Based on our experience (6543 fetal biophysical profiles over a 4-year period), the correct interpretation of the information obtained from the fetal biophysical monitoring should primarily rest on the individual biophysical components rather than on the score. This approach of considering the components rather than the overall score is an absolute necessity in order to decrease both false positive and false negative examinations. An example of a false positive examination (if the score is to be used for management) is the fetus with the biophysical profile score of 4 as determined by the presence of reactive nonstress test and normal amount of amniotic fluid. This fetus has a nonreassuring score; however, the presence of reactive nonstress test rules out fetal hypoxia, and the fact that the other biophysical components (fetal breathing movements, fetal movements) are not observed during the real-time scanning is due to normal periodicity. The low biophysical score in this case cannot be the result of fetal compromise because the nonstress test is reactive, and

based on the gradual hypoxia concept,[5, 7, 14] one of the first manifestations of fetal compromise should be nonreactive nonstress testing. In this case the overall low biophysical score should not alarm the physician, and the decision on repeat testing or delivery should be decided on the basis of a normal test result. An example of what may turn out to be a false negative examination (if the score is to be used for management) is the case of the fetus with a biophysical score of 8 or more and oligohydramnios. This fetus may be well oxygenated in the presence of normal acute markers (reactive nonstress test and/or fetal breathing present); however, cord accident is a possibility because of oligohydramnios and a vulnerable cord. If the pregnancy is at or near term this fetus should be delivered[3, 16] regardless of the reassuring score because there is a high incidence of cord accident and in utero death in spite of the evidence of good fetal condition at the time of testing, as evidenced by the presence of the other biophysical components. Although previous investigators have suggested a management protocol based on the score, the presence of oligohydramnios has been considered as an indication for delivery regardless of the overall score. In viable preterm gestations, frequent testing or delivery has been advocated depending on the individual case.[3, 7]

Management based on the biophysical profile results without considering the overall clinical context of each case. Disregarding the overall clinical context of a given case and deciding for management on the biophysical profile results alone has been a common error among physicians who utilize the fetal biophysical profile. Since there is no perfect test which, when normal, guarantees good outcome in 100% of the cases, the plan of management should consider the circumstances of each case. In our opinion, even in the presence of a normal biophysical profile result, delivery is indicated in the presence of a growth-retarded fetus with confirmed pulmonary maturity; regardless of the biophysical profile results, delivery is also indicated in postdate pregnancies with a favorable cervix or in the presence of overriding medical complications (for instance, deteriorating pregnancy-induced hypertension). The clinician should realize that the information obtained from the fetal biophysical monitoring is only a part of the entire clinical picture and should be viewed as such in the decision-making process.

Interval between testing when the previous test result is normal, equivocal, or abnormal. The interval between normal test results has in general been 1 week. As a practical rule this frequency of testing may be appropriate in certain cases. However, there are certain high-risk cases (postdate pregnancies, insulin-dependent diabetes mellitus) that are in need of more frequent testing (twice a week); this has been already recognized and suggested by some investigators.[3] In the series of Manning et al.,[3] which involved 12,620 high-risk patients managed with fetal biophysical profile assessment as the means of antepartuum fetal evaluation, there were eight perinatal deaths of structurally normal fetuses within 7 days after a reassuring biophysical assessment. Seven of these eight fetuses had test-to-death interval between 5 and 7 days, and only one had a test-to-death interval of 3 days. It is reasonable to expect that increased frequency of testing (for instance, twice a week) might have prevented some of these deaths. Based on published data[1-4] as well as our experience,[5-10] the only means to reduce the false negative rate is to further increase the frequency of testing. It is our opinion that the interval between normal tests should be individualized according to the circumstances of each case.

In cases with an equivocal or abnormal previous test result, some investigators have advocated repeat testing within 24 hours.[3] Many clinicians will test in 24 hours. We belive that retesting in 24 hours after an equivocal or abnormal test is unreasonable. A delay of 24 hours in cases of a nonreassuring fetal evaluation test has occasionally resulted in intrauterine death. This is a completely preventable castastrophe with no reasonable excuse, given the fact that the previous test was not indicative of fetal well-being. Delaying 24 hours makes no sense if the fetus is really in jeopardy; on the other hand, if the nonreassuring biophysical monitoring is the result of normal periodicity, then a repeat nonreassuring testing 24 hours later may still be the result of absent fetal activities because of normal periodicity. In our opinion there is no room for uncertainty between tests; a definite judgment about fetal condition should be made before the patient is discharged home. Repeat testing within 24 hours, after an equivocal or abnormal test result, is to be avoided; in such cases we would advocate extended testing immediately following the initial nonreassuring test result in order to differentiate the sleeping from the asphyxiated fetus and to avoid in utero deaths that may occur in the interim.

Procrastination or reluctance to act promptly on the abnormal test result. In certain situations, such as gestations with an unfavorable cervix or preterm gestations, there is a natural reluctance from many clinicians to act and pursue appropriately an abnormal test result. The clinician is frequently hesitant to induce labor in a patient with an unfavorable cervix because of the increased risk of failed induction and therefore cesarean section. However, since the clinician chooses to apply fetal evaluation even in patients with unfavorable cervix, he or she is obliged to pursue the abnormal test result rather than procrastinating because of cervical unripeness. In preterm gestations clinicians justify their

procrastination on the assumption that the biophysical activities of the fetus are not known during early gestational ages; in these preterm gestations, however, the clinician should take advantage of the fact that the sleeping cycles of the fetus are much shorter compared to those at term and therefore the incidence of false positive biophysical profile examinations is extremely low. For instance, a fetus at <30 weeks' gestation who does not exhibit any biophysical activities in a 30-minute observation period under real-time scanning is apparently asphyxiated and not sleeping. As the gestational age advances, the sleeping cycles become longer (40 to 60 minutes), and only then may an abnormal biophysical profile result be due to normal periodicity or fetal compromise. It is our opinion that an abnormal fetal biophysical result in a very preterm gestation should be pursued aggressively because it is rarely false positive. The normal fetal biophysical profile and activities in preterm gestations, as well as throughout gestation, have been recently defined by our group.[8]

Assessment of the ultrasound-monitored biophysical variables without including the nonstress test. Although it has been suggested that the addition of the nonstress test does not improve the discriminative accuracy when all ultrasound-monitored variables are normal,[3] we would not advocate this approach. In our opinion the nonstress test should be an integral part of the fetal biophysical monitoring because not only is it a sensitive indicator of fetal condition, but also, if variable decelerations are present, it may select those fetuses who are candidates for a cord accident. Since spontaneous variable decelerations can escape detection when only real-time scanning is used, it is possible to miss the occasional fetus who is a candidate for a cord accident in spite of the presence of normal amniotic fluid. The variable decelerations in these cases may be the result of a cord abnormality (short cord, true knot, etc.) or abnormal cord position (for example, tight nuchal cord). Since certain fetuses who are candidates for a cord accident will manifest spontaneous variable decelerations in the presence of normal amniotic fluid volume, we still continue to use the nonstress test as an integral part of the fetal biophysical profile assessment.

Inexperience of the operator and faulty technique. One of the most frequent errors occurs in the qualitative assessment of the amniotic fluid volume. By definition, measured pockets of amniotic fluid should be free of umbilical cord. It is imperative therefore for the operator to make sure that he does not include cord loops in his measurements. Inclusion of cord loops in the qualitative amniotic fluid assessment will as a result cause a false sense of security that it is a normal amount of amniotic fluid, whereas it may be decreased and the fetus be in jeopardy. Another area that also needs further clarification is the definition of the "normality" of the observed biophysical activities. For the purpose of antepartum fetal surveillance, fetal breathing is considered to be present only if it is continuous (breath-to-breath intervals of <6 seconds). Isolated breathing movements separated by ≥6 seconds should not be considered to be fetal breathing present. Similarly, fetal body movements are considered to be present only if rolling trunk movements are observed (three or more within 30 minutes). Isolated limb movements should not be considered to be fetal movements present. Regarding the ultrasound technique used for assessment of the fetal biophysical variables, it is reasonable to recommend that the operator use the technique with which he is most familiar. The technique that we use for the most part consists of a transverse abdominal scan with simultaneous visualization of the fetal abdomen, fetal limbs, and amniotic fluid. In this plane we measure the largest amniotic fluid pocket and also are able to simultaneously observe any breathing activity (by observing the expansion of the fetal abdominal wall), fetal body movements (as evidenced by rolling circular motion of the spine), and fetal tone (as evidenced by extension of the extremities and return to the position of flexion); very frequently the placenta is also visualized in this plane.

Proposed protocol of antepartum fetal evaluation based on information obtained from the fetal biophysical assessment

It is clear from the aforementioned discussion that the fetal biophysical profile is designed to assist the clinician to ascertain the following: (1) The fetal condition at the time of testing. This judgment should be based on the presence or absence of the acute markers of the fetal condition, namely, the nonstress test, fetal breathing movements, fetal movements, and fetal tone. (2) The degree of fetal compromise. This judgment is based on the presence or absence of the acute markers of the fetal condition interpreted according to the gradual hypoxia concept. (3) The possibility of chronic fetal stress or compromise. This judgment should be made on the basis of the amniotic fluid volume. (4) The possibility of in utero death because of cord accident. This judgment should be made based on the presence or absence of oligohydramnios and/or variable decelerations during nonstress testing. (5) Increased risk for intrapartum abnormal fetal heart rate patterns and abruptio placentae. This judgment should be based on the presence of a grade III placenta. It should also be emphasized that the fetal biophysical profile is designed to detect the presence of anomalous fetuses,[1-3, 5, 7] and this information may modify further management. The fetal biophysical profile was not designed to predict the presence or absence of other abnormal outcomes such

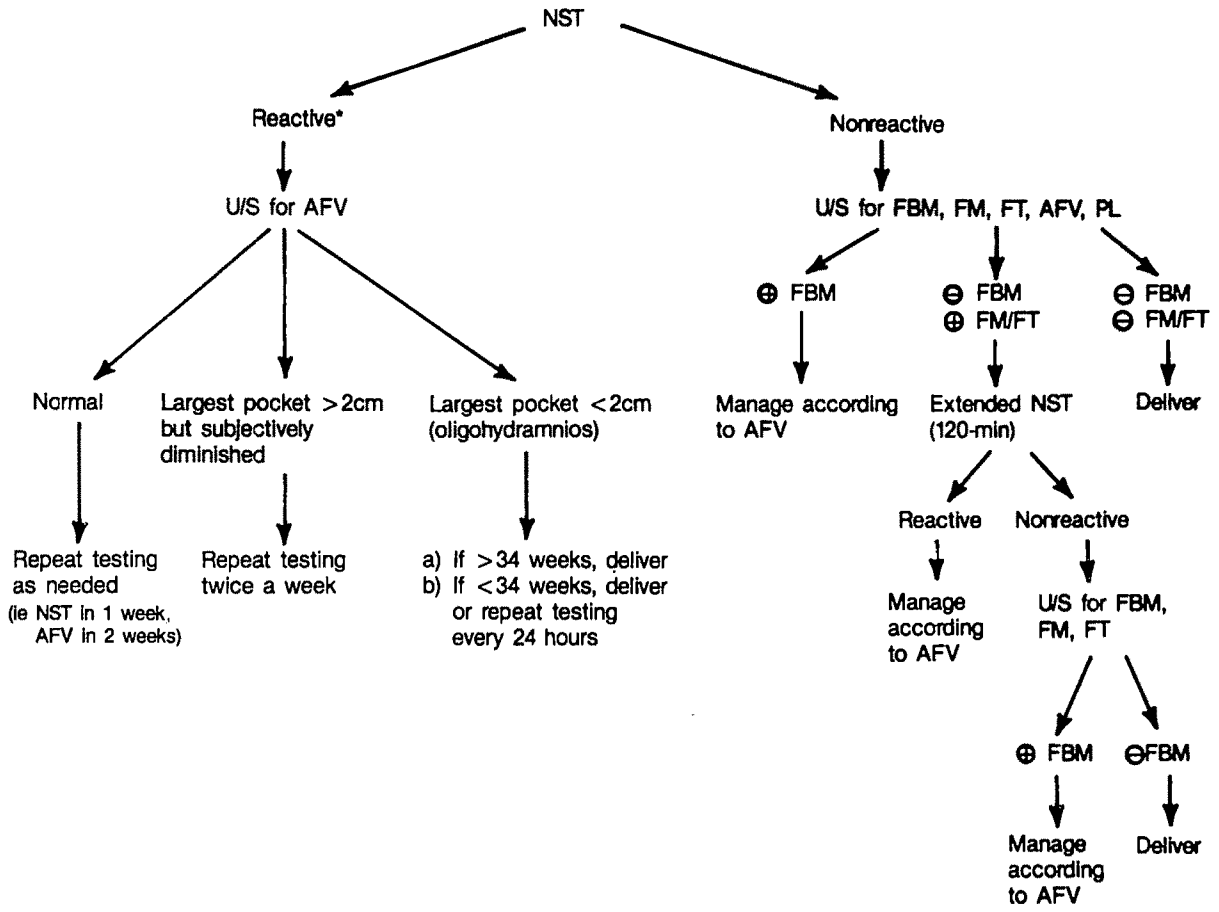


Fig. 1. Protocol of antepartum fetal evaluation for pregnancies with intact membranes. *NST* = Nonstress test, *U/S* = ultrasound, *AFV* = amniotic fluid volume, *FBM* = fetal breathing, *FM* = fetal movements, *FT* = fetal tone, *PL* = placental grading, ⊕ = present/normal, and ⊖ = absent/abnormal. *In the presence of variable decelerations and oligohydramnios consider delivery; in the presence of variable decelerations and normal *AFV* consider extended (120 minutes) or repeat *NST* within 24 hours.

as intrauterine growth retardation, postdatism, ability of the fetus to tolerate labor, meconium aspiration, premature labor, and abruptio placentae.

Considering the physiology and pathophysiology of the fetal biophysical activities as well as the erroneous judgments that could result from using the score alone and not the score components, we recommend an alternative scheme of antepartum fetal evaluation. We have applied this scheme in 6543 fetuses over a 4-year period, and we have encountered only one in utero death, which occurred 2½ weeks after a reassuring fetal biophysical assessment. When evaluating the fetus of a high-risk pregnancy, we ask three questions and the pregnancy is continued only if the fetus qualifies for normality by all three questions:

1. Is the fetus compromised at the time of testing? We recommend that a reactive nonstress test (two or more fetal heart rate accelerations in a 10-minute period) or presence of fetal breathing for >30 seconds[5-7] (even in the presence of a nonreactive nonstress test) indicate fetal well-being at the time of testing. A word of caution should be addressed at this point regarding the presence of fetal breathing as a sign of well-being in fetuses of diabetic mothers. We have encountered two hypoxic fetuses of diabetic mothers who had fetal breathing just before delivery by cesarean section, but in both cases maternal hyperglycemia was present when breathing was observed. Since maternal hyperglycemia increases fetal breathing, we would recommend that fetal breathing should be regarded as a sign of well-being only in the presence of maternal normoglycemia at the time of testing.

In the presence of a nonreactive nonstress test, real-time scanning is performed for a maximum 30-minute observation period. If fetal breathing is detected (lasting >30 seconds, the examination is terminated and further management is based on the amniotic fluid volume assessment. If all acute markers (fetal breathing, fetal movements, fetal tone) are absent or compromised after 30 minutes of continuous observation, prompt

delivery is undertaken. This action is based on the fact that these fetuses almost never improve their biophysical assessment on repeat testing because they are severely compromised.[3, 5] If the fetus has a nonreactive nonstress test and there is absence of fetal breathing but movements and tone are present, we would recommend extended nonstress testing to differentiate sleeping state from asphyxia. The nonstress testing is continued until a reactive pattern is observed or 120 minutes of continuous fetal heart monitoring have elapsed. At the end of the 120-minute period if the nonstress test is still nonreactive, the ultrasound-monitored variables (fetal breathing, fetal movements, and fetal tone) are reassessed. This is helpful in determing the degree of fetal compromise according to the gradual hypoxia concept. If after this extended testing the nonstress test remains nonreactive and no fetal breathing is observed, then delivery is considered. If the fetus is not compromised at the time of testing as evidenced by the presence of a reactive nonstress test and/or fetal breathing, the clinician should proceed with the following question.

2. Is the fetus a candidate for a cord accident? This clinical judgment is based on the presence or absence of oligohydramnios and/or variable decelerations during nonstress testing. If there are no variable decelerations and a normal amount of amniotic fluid, then testing is repeated after individualizing for each case. The presence of oligohydramnios (largest pocket, <2 cm)[5, 16] at a term or near term gestation with intact membranes is considered an indication for delivery regardless of the normality of the other fetal biophysical components because these fetuses are at high risk for cord accident and in utero death. The combination of oligohydramnios and variable decelerations during nonstress testing is an indication for delivery in viable fetuses regardless of gestational age. In a preterm gestation with intact membranes in which fetus seems to be a candidate for a cord accident (as evidenced by decreased amount of amniotic fluid or variable decelerations), individualization is recommended, and if delivery is not undertaken, testing (every 24 hours) is the plan of management. The frequency of testing should be dictated by the amount of amniotic fluid volume. In cases in which the amniotic fluid volume seems subjectively diminished but the largest amniotic fluid pocket measures >2 cm, we would recommend increased frequency of testing (twice a week). If the fetus does not seem to be a candidate for a cord accident, as evidenced by normal amniotic fluid volume and no variable decelerations, then the clinician should proceed with the following question.

3. Is the fetus growing normally? This question mainly pertains to cases of suspected intrauterine growth retardation. In our unit these fetuses are followed by serial ultrasound growth parameters every 2 weeks in addition to the fetal biophysical monitoring. If there is normal rate of growth and normal biophysical assessment, the pregnancy is allowed to continue. However, if there is suboptimal or arrested rate of growth at term or near term, delivery is undertaken. If delayed growth rate is diagnosed in a preterm gestation, frequent biophysical monitoring is indicated.

The protocol of antepartum fetal surveillance for pregnancies with intact membranes, used at the authors' institution, is illustrated in Fig. 1. We believe that this alternative protocol of antepartum fetal surveillance, without including the scoring of the biophysical variables, will eliminate most of the problems encountered in the clinical interpretation and application of fetal biophysical monitoring. The understanding of the physiology, pathophysiology, and the gradual hypoxia concept of the fetal biophysical activities, as well as the significance of oligohydramnios, is imperative for the proper clinical application of this tool and therefore for the improvement of the quality of perinatal care.

REFERENCES

1. Manning FA, Plat LD, Sipos L. Antepartum fetal evaluation: development of a fetal biophysical profile score. Am J Obstet Gynecol 1980;136:787.
2. Manning FA, Morrison I, Lange IR. Fetal biophysical profile scoring: a prospective study in 1184 high-risk patients. Am J Obstet Gynecol 1981;140:289.
3. Manning FA, Morrison I, Lange IR. Fetal assessment based on fetal biophysical profile scoring: experience in 12,620 referred high-risk patients. I. Perinatal mortality by frequency and etiology. Am J Obstet Gynecol 1985;151:343.
4. Schifrin BS, Guntes V, Gergely RC, et al. The role of real-time scanning in antenatal fetal surveillance. Am J Obstet Gynecol 1981;140:525.
5. Vintzileos AM, Campbell WA, Ingardia CJ, et al. The fetal biophysical profile and its predictive value. Obstet Gynecol 1983;62:271.
6. Vintzileos AM, Campbell WA, Nochimson DJ, et al. The fetal biophysical profile in patients with premature rupture of the membranes: an early predictor of fetal injection. Am J Obstet Gynecol 1985;152:510.
7. Vintzileos AM, Campbell WA, Nochimson DJ, et al. The use of real-time scanning in antepartum fetal evaluation: the fetal biophysical profile. In: Sanders RC, Hill MC, eds. Ultrasound annual 1985. New York: Raven Press, 1985:251.
8. Vintzileos AM, Feinstein SJ, Lodeiro JG, et al. Fetal biophysical profile and the effect of premature rupture of the membranes. Obstet Gynecol 1986;67:818.
9. Vintzileos AM, Campbell WA, Nochimson DJ, et al. Fetal biophysical profile versus amniocentesis in predicting infection in preterm premature rupture of the membranes. Obstet Gynecol 1986;68:488.
10. Lodeiro JG, Vintzileos AM, Feinstein SJ, et al. The fetal biophysical profile in twin gestations. Obstet Gynecol 1986;67:824.
11. Platt LD, Walla CA, Paul RH, et al. A prospective trial of the fetal biophysical profile versus the nonstress test in the management of high-risk pregnancies. Am J Obstet Gynecol 1985;153:624.
12. Patrick J, Challis J. Measurement of human fetal breath-

ing movements in healthy pregnancies using a real-time scanner. Semin Perinatol 1980;4:275.
13. Patrick JE, Featherson W, Vick H, et al. Human fetal breathing and gross body movements at weeks 34 to 35 of gestation. AM J OBSTET GYNECOL 1978;130:693.
14. Humphrey T. Function of the nervous system during prenatal life. In: Uwe S, ed. Perinatal physiology. New York: Plenum, 1978:651.
15. Ianniruberto A, Tejani E. Ultrasonographic study of fetal movements. Semin Perinatol 1981;5:175.
16. Chamberlain PF, Manning FA, Morrison I, et al. Ultrasound evaluation of amniotic fluid volume. I. The relationship of marginal and decreased amniotic fluid volumes to perinatal outcome. AM J OBSTET GYNECOL 1984;150:245.
17. Seeds AE. Current concepts of amniotic fluid dynamics. AM J OBSTET GYNECOL 1980;138:575.

Maternal serum α-fetoprotein screening

II. Pitfalls in low-volume decentralized laboratory performance

James N. Macri, Ph.D., Ramana V. Kasturi, B.E., M.S., David A. Krantz, B.S., and Mannshya G. Hu, Ph.D.

Carle Place, New York

Assays of maternal serum α-fetoprotein are subject to the phenomenon of assay drift, which may be defined as incorrect increase or decrease of α-fetoprotein values from their true values. Low maternal serum α-fetoprotein weekly volume (for example, fewer than 500 specimens per week) will result in a >47% probability that 10% assay drift will not be recognized. Further, laboratory reports to clinicians may lead to either misdirecting 43% more pregnant women (with positive drift) into further (possibly invasive) diagnostic procedures or the offer of further diagnostic services to 32% fewer gravidas at increased risk (with negative drift) than should be so managed. We address the problem outlined above and present the reasons for establishment of regional maternal serum α-fetoprotein screening programs operating at sufficient volume to permit the identification and control of assay drift. (AM J OBSTET GYNECOL 1987;156:533-5.)

Key words: Maternal serum α-fetoprotein, assay drift, low volume

Maternal serum α-fetoprotein screening for the prenatal detection of fetal malformations and genetic anomalies is increasingly used in the United States. Because of the structure of health care delivery and the number of clinical laboratories it is possible that this unprecedented, population-based screening process will be provided in a decentralized fashion.

Strong influence to decentralize maternal serum α-fetoprotein screening may be exerted by laboratories situated in hospitals and medical centers. Decentralized, low-volume maternal serum α-fetoprotein screening should be of considerable concern to obstetricians and geneticists. We set forth the reasons for concern and recommend that regional, joint programs be established for safe and effective maternal serum α-fetoprotein screening.

Since it is unlikely that most hospitals or medical centers will care for >3000 obstetric patients per year, small, decentralized maternal serum α-fetoprotein screening programs will be evaluating approximately 12 or fewer patients per day. At such levels of screening, laboratories will be required to use more reagents for standards and controls than for patient specimens. The unfavorable cost effects are obvious. It is inevitable that economic pressure will lead to batching of specimens, increased turnaround time, delayed information flow to physicians and patients, and detriment to the purpose and objectives of maternal serum α-fetoprotein screening.

Few clinical laboratories in the United States have maternal serum α-fetoprotein screening experience. However, many laboratories are permitted to proceed without first having developed their own, independent, normative data and while performing maternal serum α-fetoprotein assays on a low-volume basis.[1] Under such conditions the likelihood of assay imprecision will be great. For example, the report of a workshop sponsored by the National Institute of Child Health and Human Development[2] implies that laboratories evaluating 50 patient specimens per week would require recurring

From the Research Division, Neural Tube Defect Laboratories.
Reprint requests: James N. Macri, Ph.D., Neural Tube Defect Laboratories, 383 Old Country Road, Carle Place, NY 11514.

Table IA. Probability (expressed as percent) that a given positive assay drift will be undetected at various assay sizes*

No. of patients per assay	*Positive assay drift (%)*						
	+20%	*+17.5%*	*+15%*	*+12.5%*	*+10%*	*+7.5%*	*+5%*
30	42.07	—†	—	—	—	—	—
50	22.96	32.64	46.04	—	—	—	—
100	4.27	9.18	17.88	31.21	48.01	—	—
150	0.68	2.28	6.55	16.11	32.28	—	—
200	<0.1	0.51	2.22	7.78	21.19	43.64	—
250	<0.1	0.11	0.73	3.67	13.35	34.46	—
300	<0.1	<0.1	0.23	1.66	8.23	27.09	—
350	<0.1	<0.1	<0.1	0.73	5.05	21.19	—
400	<0.1	<0.1	<0.1	0.32	3.07	16.35	48.01
450	<0.1	<0.1	<0.1	0.13	1.79	12.51	43.64
500	<0.1	<0.1	<0.1	<0.1	1.04	9.51	39.36
550	<0.1	<0.1	<0.1	<0.1	0.60	7.08	35.20
600	<0.1	<0.1	<0.1	<0.1	0.35	5.37	31.56
1000	<0.1	<0.1	<0.1	<0.1	<0.1	0.45	12.30

*Based on type II error associated with hypothesis testing at a level of significance of 0.05.
†Designated percent of assay drift falls within acceptable limits.

Table IB. Probability (expressed as percent) that a given negative assay drift will be undetected at various assay sizes*

No. of patients per assay	*Negative assay drift (%)*						
	−20%	*−17.5%*	*−15%*	*−12.5%*	*−10%*	*−7.5%*	*−5%*
30	38.21	—†	—	—	—	—	—
50	13.35	26.11	42.07	—	—	—	—
100	0.49	2.94	10.75	26.43	47.61	—	—
150	<0.1	0.22	2.07	10.03	28.77	—	—
200	<0.1	<0.1	0.34	3.44	16.35	42.47	—
250	<0.1	<0.1	<0.1	1.07	8.69	32.28	—
300	<0.1	<0.1	<0.1	0.31	4.55	23.89	—
350	<0.1	<0.1	<0.1	<0.1	2.28	17.62	—
400	<0.1	<0.1	<0.1	<0.1	1.10	12.71	48.01
450	<0.1	<0.1	<0.1	<0.1	0.52	9.01	42.86
500	<0.1	<0.1	<0.1	<0.1	0.24	6.30	38.21
550	<0.1	<0.1	<0.1	<0.1	0.11	4.36	33.72
600	<0.1	<0.1	<0.1	<0.1	<0.1	3.01	29.81
1000	<0.1	<0.1	<0.1	<0.1	<0.1	0.12	10.03

*Based on type II error associated with hypothesis testing at a level of significance of 0.05.
†Designated percent of assay drift falls within acceptable limits.

blocks of time (3 months) and recurring levels of patient volume (600 patients) to be able to assess the quality of assays performed during the 3-month period. These conditions and time lag are incompatible with the best interests of clinicians and patients, who will have already passed critical points of decision-making.

At low-volume levels the intrinsic problems of assay drift could be difficult, if not impossible, to monitor in a timely fashion (before physician receipt of report). The private and public proficiency testing programs that are appearing are not likely to solve for clinicians the inherent potential for inaccuracy which we describe.

Information from proficiency testing programs indicates that even experienced maternal serum α-fetoprotein laboratories will experience assay drift.[3] Unrecognized assay drift will lead to a significant problem manifesting itself in at least two ways: (1) wide differences in the number of patients (among maternal serum α-fetoprotein screening programs) falling into risk categories warranting further evaluation as well as (2) unexplained differences in detection efficiencies between maternal serum α-fetoprotein screening programs. The problem will have negative clinical implications.

Assay drift may be monitored by taking advantage of the central limit theorem, which states that for large volumes of specimens, the mean of the sample is nor-

Table II. Effect of assay drift on number of pregnant women receiving further evaluation*

	No. of pregnant women above cut-off	*No. of pregnant women inappropriately above or below cut-off*	*% of change*
With no drift	44	0	0
With positive drift of (%)			
5	53	9	20
7.5	58	14	32
10	63	19	43
12.5	69	25	57
15	76	32	73
17.5	85	41	93
20	93	49	111
With negative drift of (%)			
5	36	8	−18
7.5	33	11	−25
10	30	14	−32
12.5	27	17	−39
15	25	19	−43
17.5	23	21	−48
20	21	23	−52

*Hypothetical program using 2.5 multiples of the median cut-off in screening 1000 patients.

mally distributed about the population mean. By calculating the mean multiple of the median* for all samples on an individual assay and accepting or rejecting the assay based on whether this mean multiple of the median is within or outside an established range (95% confidence interval), laboratories will be capable of detecting and correcting for assay drift. When an assay is accepted and results are subsequently reported to physicians, there remains the concern that assay drift may have occurred, notwithstanding the fact that the mean multiple of the median for that assay is within the established limits for accepting the individual assay.

Tables IA and IB represent such probabilities based on data established by us from experience in the evaluation of α-fetoprotein from approximately a quarter million pregnancy samples. At an assay volume of 50 specimens the probability of erroneously accepting an assay with a positive 15% drift is in the range of 46%. In this same 50-specimen assay 12.5% drift falls within acceptable limits.

Table II illustrates that, with positive assay drift, more than the accurate number of pregnancies will fall above specified cut-off levels and would unnecessarily receive further, possibly invasive testing. A 5% positive drift would result in 20% of additional pregnant women (53 compared to 44) falling above specified cut-off levels. A 20% positive drift will result in 111% additional pregnant women (93 compared to 44) falling above specified cut-offs. Similarly, negative assay drift will result in erroneously assuring some pregnant women that no further evaluation is necessary.

Unrecognized, positive assay drift resulting in the misdirection of (added numbers) pregnant women to potentially invasive procedures will have a calculable effect on fetal harm. Similarly, unrecognized negative assay drift will result in sharp differences among maternal serum α-fetoprotein screening programs in detection efficiencies for neural tube defects. Highly variable levels of accuracy in maternal serum α-fetoprotein screening will result (and be made available to physicians) from the failure to develop independent normative data and the failure to perform high volume and adequately monitored assays.

*We note that use of multiples of the median as statistical parameters of measurement are appropriate, but they should not be used for reporting to clinicians or patients.

REFERENCES

1. Committee report: guidelines to assist the quality of systems for alpha-fetoprotein (AFP) assay in prenatal diagnosis. Villanova, Pennsylvania: National Committee for Clinical Laboratory Standards publication; vol 1, No. 3, 1981.
2. Wald NJ, Cuckle HS. The quality control of alpha-fetoprotein reagents and assay for the antenatal screening and diagnosis of open neural tube defects: report of a workshop sponsored by the National Institute of Child Health and Human Development (NICHHD). Clin Chim Acta 1980; 105:9-24.
3. Knight GJ, Haddow JE, Palomaki G. An external laboratory quality control program to help ensure reliability for maternal serum alpha-fetoprotein screening. Scarborough, Maine: Foundation for Blood Research, January 29, 1985.

Prevention of preterm birth by ambulatory assessment of uterine activity: A randomized study

John C. Morrison, M.D., James N. Martin, Jr., M.D., Rick W. Martin, M.D., Kathy S. Gookin, R.N., M.S.N., and Winfred L. Wiser, M.D.
Jackson, Mississippi

Tocodynamometry, used on an ambulatory basis, has been shown to detect uterine activity. The objective of this study was to assess the effectiveness of ambulatory tocodynamometry in the early identification of preterm labor. In this investigation 67 women at risk for preterm labor were randomly divided into two groups: 34 received a uterine activity monitor while 33 patients used palpation. Approximately two thirds of the study sample developed preterm labor. Upon diagnosis of preterm labor, parturients in the control group had cervical dilatation of <3 cm ($p < 0.001$) and effacement of >50% more often than the monitored group ($p < 0.01$). As a result there was a significant decrease in the number of patients who responded to tocolytic therapy in the unmonitored group. Among those with preterm labor the time gained in utero was greater in the monitored group (8.2 ± 2.7 weeks) compared to the control group (4.2 ± 2.9 weeks) ($p < 0.05$). Subsequently 29 of 34 monitored patients attained term (36 completed gestational weeks) versus only 18 of the 33 patients in the unmonitored group ($p < 0.01$). Although the sample size is relatively small, uterine activity monitoring in these very high risk patients resulted in an increased number of suitable candidates for tocolysis and allowed a significantly greater percentage of women to reach term. (Am J Obstet Gynecol 1987;156:536-43.)

Key words: Preterm, ambulatory detection, uterine activity

Although there has been tremendous improvement in perinatal morbidity and mortality in the past 20 years, the percent of parturients delivering preterm infants in the United States has remained largely unchanged.[1] In addition, approximately 75% of neonatal mortality is related to respiratory problems that are causally associated with prematurity. Methods of preventing preterm birth have been impaired because the exact etiology of preterm labor has not been delineated.[2] Tocolytic agents are effective in delaying preterm births in selected cases, but in many patients therapy is not salutary because labor is not detected before advanced dilatation of the cervix.[3] Recently, Herron et al.[4] have developed a method of addressing preterm birth that involves identification of high-risk patients as well as educational intervention toward prevention of early delivery. This schema purports to allow earlier identification of labor and thus more timely medical treatment. Unfortunately, many times uterine activity is not perceived by the patient.[5] Also, other studies using the technique of intensive risk assessment and patient education in a socioeconomically disadvantaged population did not find this technique successful in preventing preterm birth.[6]

Bell[7] has demonstrated that increased uterine activity as measured by tocodynamometry can be accurately detected in the preterm parturient. More recently, Katz and Gill[8] have shown that the use of a uterine activity monitoring device in ambulatory patients at home could accurately detect uterine activity. Previous studies involving this device have been successful in predicting uterine activity and preterm labor.[5, 9] The objective of this investigation was to determine in a randomized study whether ambulatory assessment of uterine activity with use of the Term Guard system (Tokos Medical Corp., Santa Ana, California) would aid in the diagnosis

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Mississippi Medical Center.
Supported in part by the Vicksburg Hospital Medical Foundation.
Presented at the Fifty-fourth Annual Meeting of the Central Association of Obstetricians and Gynecologists, Milwaukee, Wisconsin, October 10-12, 1986.
Received for publication August 6, 1986; revised October 3, 1986; accepted October 10, 1986.
Reprint requests: John C. Morrison, M.D., Department of Obstetrics and Gynecology, University of Mississippi Medical Center, 2500 North State St., Jackson, MS 39216-4505.

of preterm labor at an earlier time when tocolytic agents might be more effective in prolonging gestations to term.

Material and methods

Over a 9-month period women in the University of Mississippi Medical Center Obstetric Clinic were assessed for factors placing them at high risk for preterm delivery. These factors included multifetal gestations, uterine abnormalities, and the history of two or more previous preterm deliveries. Patients with historic events associated with a definite but lower risk of preterm labor (such as uterine irritability, second-trimester abortion, cervical incompetence, diethylstilbestrol exposure) were not candidates for this study. Seventy-five women >14 and <24 weeks' gestation were identified for the prospective randomized trial. All patients attended the High Risk Preterm Labor Clinic and were managed by house officers who received supervision from attending staff. Of the 75 women, six refused to enroll in the study because of the requirement of randomization. After an informed consent form was signed, the 69 remaining patients were randomized into one of two groups based on the last digit of their hospital number: those with odd numbers received the monitor and ones with even numbers were monitored by self-palpation. Two patients who enrolled were removed from the study; one aborted before the twentieth week and one moved from the area and was not available for study. This left 67 patients in the sample: 34 in the monitored group and 33 in the control group.

Subjects in both groups were given instruction regarding early signs and symptoms of preterm labor and were taught techniques of self-monitoring of uterine activity.[9] Patients were told to come to the hospital if they developed any symptoms of preterm labor (pelvic heaviness, increased vaginal discharge, back pain, rupture of the membranes, or palpable uterine contractions). All parturients were given a toll-free telephone number in the obstetric unit that was answered 24 hours each day and told to call if any questions regarding preterm labor or other aspects of pregnancy occurred. Subjects in the nonmonitored group were contacted by phone at least twice per week to assess uterine activity by palpation and patient well-being. None of the patients were told to avoid intercourse or limit their daily activities. In both groups the subjects were assessed by a gentle cervicovaginal examination every 2 weeks.

Those in the monitored group received a tocodynamometry device (Term Guard) developed for monitoring and storage of uterine activity data in the ambulatory patient. Each patient in the monitored group was instructed concerning the operation of the device and was told to monitor for 1 hour each evening and 1 hour in the morning. After monitoring the stored data were transmitted (usually in the morning) via telephone to a receiving tocograph in the center. If other symptoms (as outlined above for the control group) occurred, the patients were told to monitor for 1 hour and contact the study nurse. This device allowed ambulation during monitoring and has previously been shown to be reliable in recording even mild contractions that occur during the period of early labor.[8]

Each transmission time was approximately 6 minutes for the 120 minutes of stored data. After transmission a baseline of uterine activity was established, which usually averaged zero to three contractions per hour in each patient. If four or more contractions per hour, each lasting ≥40 seconds, were noted, the patient was asked to assume the recumbent position (left side), take hydration orally, and remonitor for an hour. If the excess uterine activity remained unabated, she was instructed to come to the hospital obstetric receiving area where a cervical examination and assessment for labor was performed.

In both groups if preterm labor was suspected, patients were admitted to the hospital receiving area and were observed by staff members unaware of the subjects' involvement in the study. If uterine activity was accompanied by progressive cervical change, a parenteral tocolytic agent was administered. These objective criteria for tocolytic therapy followed those of Zahn[10] and were applied to patients in both groups. In no subjects were uterine relaxants administered prophylactically or without cervical change. The tocolytic agent used was most often magnesium sulfate; in a few patients, ritodrine hydrochloride was employed. However, the frequency of use for either drug was equally distributed in both groups of patients.

If tocolysis was successful, patients were discharged on oral agents, bed rest (except for meals and bathroom privileges), and more frequent prenatal visits. The dosage of tocolytic drugs was adjusted to attain three or less contractions per hour in the monitored group and to abolish perceived contractions in control patients. Oral tocolysis and uterine activity monitoring were discontinued at 36 completed weeks' gestational age. Other parameters of care (particularly monitoring or nonmonitoring) remained the same as before treatment. For the purposes of this study, 36 completed gestational weeks were considered term. Pregnancies terminated before that time for reasons other than preterm labor were defined as medically indicated deliveries. Gestational age assessment was based on menstrual data, ultrasonography, and neonatal evaluation.

Statistical analysis was carried out by the use of χ^2 analysis and the Student t test. A value of $p < 0.05$ was used to designate statistical significance. Data were ex-

Table I. Patient population

Risk factor	*Control group*	*Monitored group*
Previous preterm birth (n)	22	20
Multifetal gestation (n)	8	10
Uterine abnormality (n)	3	4
Total	33	34

Table II. Demographic characteristics

Maternal factors	*Control group*	*Monitored group*
Maternal age (yr)	23.2 ± 3.9	22.9 ± 4.1
Ethnic group		
Black (n)	25	26
White (n)	7	6
American Indian (n)	1	2
Parity	2.8 ± 0.9	2.6 ± 0.7
Weeks' gestation at initial contact	18.4 ± 2.6	18.6 ± 3.2

pressed as the mean ± the standard error of the mean.

Results

The distribution of risk factors for preterm birth was similar between the two groups (Table I).

As shown in Table II, there was no statistically significant differences in maternal age, ethnicity, or parity between the control or the monitored group. In addition, at the initial prenatal contact the patients in both groups were at a similar stage of gestation.

Of the 33 patients in the control group 22 (67%) had documented preterm labor. Of these, 15 (68%) had advanced cervical dilatation (3 cm or more) and 14 (64%) were more than 50% effaced at the time of diagnosis (Table III). In contradistinction, of the 24 (71%) patients who had preterm labor in the monitored group, only two (8%) had a cervical dilatation of ≥3 cm, and six (25%) had cervical effacement of >50% at diagnosis of preterm labor. The differences between the monitored and nonmonitored patients in cervical dilatation ($p < 0.001$) and effacement ($p < 0.01$) were statistically significant. In addition, the frequency of contractions among monitored patients who developed preterm labor was significantly increased during the last 24 hours before admission when compared to uterine activity in those who did not have preterm labor. Likewise, there were five of 22 (23%) patients in the control group who had ruptured membranes as compared to one of 24 (4%) in the monitored group.

With use of the Baumgarten Tocolytic Index[11] upon admission for preterm labor, 10 of 22 (45%) in the control group compared to 22 of 24 (92%) in the monitored group were considered to be good candidates for long-term tocolysis ($p < 0.001$) (Table IV). Although all subjects in both groups with preterm labor were treated with tocolytic agents, of those thought to have unfavorable conditions for long-term tocolysis (cervix ≥3 cm and >50% effaced), 10 unmonitored patients and one monitored patient delivered within 96 hours of admission, thus validating this method of selecting patients for tocolysis.[11, 12] Among subjects thought to be suitable for long-term tocolysis (cervix ≤2 cm and <50% effaced) in both groups, three of 10 controls and one of 22 monitored patients delivered within 1 week in spite of tocolytic therapy. Also the number of medically indicated deliveries was equal in each group (Table IV). Finally, among all patients only slightly more than half with documented preterm labor perceived their contractions.

Table III. Maternal factors at diagnosis of preterm labor

Factor	*Control group*	*Monitored group*	*p*
Preterm labor (n)	22	24	
Cervical dilation (n)			
≤2	7	22	<0.001
≥3	15	2	
Cervical effacement (n)			
<50%	8	18	<0.01
>50%	14	6	
Rupture of membranes (n)	5	1	<0.06

Monitoring in the patients with use of the tocodynamometer began at an average of 25.9 ± 2.8 weeks' gestation (Table V). This monitoring continued for 9.5 weeks (±4.1 weeks). The gestational age at which diagnosis of preterm labor was made was earlier in the monitored group (27.9 ± 2.4 weeks) versus the control group (29.4 ± 2.7 weeks). These results are not statistically different because of small sample size but approached significance ($p < 0.08$). Only 18 of the 33 (55%) patients reached 36 completed gestational weeks in the control group compared to 29 of 34 (85%) in the monitored patients ($p < 0.01$). The time gained in utero from the point of diagnosis of preterm labor was also greater in the monitored group, being 8.2 ± 2.7 weeks when compared to the control group of 4.2 ± 2.9 weeks ($p < 0.05$).

Comment

Neonatologists in their care of the low birth weight neonate have decreased morbidity and mortality but are ultimately limited by the immaturity of the infant.[1] Therefore the most critical area of concentration for perinatologists is the reduction in frequency of preterm birth. One of the most notable advances in the prevention of preterm birth has been the identification of

Table IV. Outcome data

	Control group	*Monitored group*
Suitable tocolysis candidate*	10/22	22/24
Failed tocolysis†	3/10	1/22
Indicated deliveries (n)	2	2
Perceived contractions (n)	12	13

*Cervix ≤2 cm, <50% effaced; all patients in both groups received tocolysis.

†Delivered within 1 week despite being suitable tocolytic candidate.

Table V. Outcome data

	Control group	*Monitored group*
Gestational age (wk)		
Start of monitoring	—	25.9 ± 2.8
Weeks monitored	—	9.5 ± 4.1
Gestational age at diagnosis (wk)	29.4 ± 2.7	27.9 ± 2.4
Delivered ≥37 wk	18	29
Time gained in utero (wk)*	4.2 ± 2.9	8.2 ± 2.7

*From time of diagnosis of preterm labor.

patients at risk for this problem.[2, 4] One of the difficulties, however, associated with risk assessment for preterm birth is its poor sensitivity whereby a significant percentage of the preterm births occur in those without identifiable risk factors. Also, parturients do not always appreciate subtle uterine activity even after education about signs and symptoms of preterm labor.[5] Therefore, regardless of the education and frequent cervical examinations, many women do not perceive contractions or prodromal labor signs[6, 13] and are likely to present as unsuitable candidates for long-term tocolysis.

Often the provider cannot inhibit labor with tocolytic agents because the cervix is already too dilated and effaced for tocolysis to be effective.[3, 12] It has been pointed out that if the cervix is ≥3 cm dilated and <1 cm long, there is a 50% reduction in efficacy of tocolytic agents in postponing delivery.[11] Likewise, the incidence of rupture of the membranes increases as the cervix dilates.[3, 5] The current study confirms that early detection of preterm labor offers better candidates for tocolysis. A significantly larger proportion of women in the monitored group were treated earlier (in terms of cervical dilatation, effacement, and rupture of the membranes) when compared to the control group, although the incidence of preterm labor was similar in both groups. In addition, there was similarity in that only half of the women with preterm labor in both groups appreciated that they were having uterine activity. This probably accounts for the significant decrease in the number of women benefiting from long-term tocolysis in the control group when compared to the monitored women who were referred for treatment earlier even though they did not feel the contractions.

The outcome data in both groups also revealed a significant increase in the number of pregnancies carried to term when ambulatory uterine activity monitoring was used. In addition, the time gained in utero was nearly doubled for monitored patients when compared to unmonitored ones (Table V). The value of uterine activity monitoring in providing more reasonable tocolytic candidates appears to be the major factor in the increased number of patients reaching 36 completed gestational weeks. We are aware that daily phone contact and subsequent reassurance in the monitored patients could have contributed to the number reaching term. Those in the unmonitored group were also contacted but only twice each week. The variability of phone contact (daily versus biweekly) could be a confounding variable because increases in uterine activity should precede clinical symptoms by 24 to 48 hours.[5, 13] Nevertheless, since there is poor sensitivity by palpation and because the control patients were instructed to call the center if early signs of labor ensued, we feel it is unlikely that the more frequent nurse-patient phone contact alone greatly influenced the outcome of this study. Likewise, the enrollment criteria were very strict in this study. Undoubtably this contributed to the high incidence of preterm labor in our patients. This finding underscores the need for stringent selection criteria for those using this detection modality so that the greatest impact on preterm birth can be attained while using this device only in appropriate patients.

In summary, although the numbers are relatively small, the data in this study would point out that greater time in utero, a greater number of suitable candidates for tocolysis, and most importantly, a reduction of preterm deliveries were noted in the monitored group. This is particularly important because the patients were randomized and were comparable for the high-risk factors for preterm birth as well as for demographic statistics. It would appear that the Term Guard system is an effective method to detect preterm labor and prevent preterm delivery.

REFERENCES

1. Quilligan EJ, Little AB, Oh W, et al. An evaluation and assessment of the state of the science. In: Pregnancy, birth and infant. Bethesda, Maryland: National Institutes of Health, 1983; NIH publication no. 82-02304.
2. Gonik B, Creasy RK. Preterm labor: its diagnosis and management. AM J OBSTET GYNECOL 1986;154:3.
3. Zlatnik FJ. The applicability of labor inhibitors to the problem of prematurity. AM J OBSTET GYNECOL 1972; 113:704.

4. Herron MA, Katz M, Creasy RK. Evaluation of a preterm birth prevention program: preliminary report. Obstet Gynecol 1982;59:452.
5. Newman RB, Gill PJ, Wittreich P, et al. Maternal perception of prelabor uterine activity. Obstet Gynecol 1986; 68:765.
6. Main DM, Gabbe SG, Richardson D, et al. Can preterm deliveries be prevented? AM J OBSTET GYNECOL 1985; 151:892.
7. Bell R. The prediction of preterm labour by recording spontaneous antenatal uterine activity. Br J Obstet Gynaecol 1983;90:884.
8. Katz M, Gill PJ. Initial evaluation of an ambulatory system for home monitoring and transmission of uterine activity data. Obstet Gynecol 1985;66:273.
9. Katz M, Gill PJ, Newman RB. Detection of preterm labor by ambulatory monitoring of uterine activity for the management of oral tocolysis. AM J OBSTET GYNECOL 1986; 154:1253.
10. Zahn V. Uterine contractions during pregnancy. J Perinatol Med 1984;12:107.
11. Baumgarten K, Gruber W. Tokolyseidex. In: Dudenhausen JW, Saling E, eds. Perinatal Medizen. Stuttgart: George Thieme Verlag. 1974:58.
12. Richter R. Evaluation of success in treatment of threatening premature labor by betamimetic drugs. AM J OBSTET GYNECOL 1977;127:482.
13. Katz M, Newman RB, Gill PJ. Assessment of uterine activity in ambulatory patients at high risk of preterm labor and delivery. AM J OBSTET GYNECOL 1986;154:44.
14. Papiernik E, Bouyer J, Dreyfus J, et al. Prevention of preterm births: the perinatal study of Haguenau. Pediatrics 1985;76:154.

Discussion

DR. FEDERICO G. MARIONA, Detroit, Michigan. It is generally accepted that there are solid data to provide evidence that perinatal outcome has improved in the last 20 years. However, the frequency with which preterm birth occurs in this country continues unabated at a rate of nearly 8%. Delivery before 37 completed gestational weeks is tantamount to a manyfold risk for neonatal death.

Dr. Morrison and his associates attempted to demonstrate that it is possible to identify uterine hyperactivity in the ambulatory pregnant patient who carries an increased risk for preterm labor. This identification would lead to early treatment and reduction of preterm births. Indeed it is a simple and straightforward plan. One must rapidly realize, however, that the sequence that stimulates the initiation of labor in the woman is still poorly understood and uterine hyperactivity represents only one prime mover.

One of the frequent variables that alters the clinician's ability to effectively assess the patient's risk is the timing of entering into prenatal care. The population studied began care early in pregnancy (approximately 20 weeks' gestation), demonstrating that there was a clear effort to reduce the barriers to prenatal care, one important factor in effective risk assessment that must not be overlooked.

I suggest that the study is supplemented by an attempt to define endocervical bacteriology in the population at risk. In this manner another potential source of risk can be studied.

Some aspects of the study are loosely described; such as, what were the sensitivity and the specificity of the methods used in the diagnosis of preterm labor? How many visits to the obstetric unit were necessary after the program was initiated until the actual diagnosis was made?

It is interesting that a team participating in the evaluation of these patients used "intensive physical assessment" with "gentle" pelvic examinations every 2 weeks initially and more frequent visits once the patients needed tocolytic therapy. Can the authors comment on the patient's acceptance of such examinations and perhaps on the potential vagaries of using a number of house officers in the cervical evaluation, unless they were able to avoid the usual "July crisis"?

The use of bed rest, with ambulation allowed for meals and bathroom privileges, appears to be a variable that may merit future consideration. Ambulatory monitoring may not mean staying home in bed, but, if so, its effects may need separate consideration, including the thorny issue of patient compliance.

Conversely, this bed rest may be offset by more frequent visits and perhaps more vaginal examinations. The authors noted that in patients who were clinically diagnosed as candidates for long-term tocolysis, 30% of the control group and 4.5% of the monitored group were delivered before term in spite of similar clinical conditions and therapy. Are there any thoughts as to why this was so?

The patient's inability to detect the presence of contractions in more than 50% of the cases is again emphasized. Perhaps the process of education should be partially modified to place a greater index of suspicion on low pelvic pressure, back pain, changes in vaginal discharge, etc., as these have been found in our own experience, as opposed to placing a heavy emphasis on "contractions."

The authors also emphasized that intercourse was allowed to continue unrestricted. Was this advice changed after long-term tocolysis was initiated? No adolescents were entered in the study. This may reflect the characteristics of the authors' population or an effort to avoid this particular group. Indeed in our experience they represent a very special patient population who generate a number of preterm births. I will certainly expect to see some of them included in the next report.

The authors accomplished their stated goal. Uterine hyperactivity was detected earlier in the monitored group, yielding earlier access to effective tocolytic therapy and ultimately a better outcome represented by a significant difference in prolongation of gestation.

In this era of diagnosis-related groups and cost-effectiveness studies, this work represents an ideal example for a comparison of the cost of home uterine activity monitoring, including the sporadic hospitalizations, long-term therapy, missed workdays, and eventually extra help in the house versus the investment of short- and long-term caring for preterm infants. I am convinced that a relevant issue can be raised to dem-

onstrate the clear advantage of this program. Serious social implications are involved and it may even be possible to obtain the politician's attention to this issue.

If perinatologists are to make a significant change in the present rate of preterm birth and the associated perinatal loss, postponing delivery and maintaining the fetus in a safe environment until the thirty-seventh completed gestational week are the ultimate goals. We owe this effort to the next generation.

DR. WASHINGTON C. HILL, Omaha, Nebraska. Dr. Morrison and his colleagues have reported the first prospective and randomized study on the prevention of preterm birth by ambulatory assessment of uterine activity. Although home tokodynamometry has been shown in a controlled study to be of benefit, these investigators are the first to show in a prospective and randomized manner that patients who are at increased risk for preterm labor and are using ambulatory monitoring were more suitable candidates for tocolysis, had their pregnancies maintained longer, had a decreased number of preterm births, and required a fewer number of maternal and neonatal hospitalization days. Why is this important? As pointed out by Dr. Morrison, the most significant problem we as obstetricians have yet to conquer in perinatal medicine is the detection of preterm labor and prevention of preterm birth. The results of studies are controversial as to whether this can be done alone by the techniques of intensive risk assessment and patient education. The authors have taken, for the most part, a socioeconomically disadvantaged group and randomized them into those who used home tokodynamometry and those who did not. Both groups had close contact with a nurse and patient education. The monitoring of uterine activity to detect preterm labor is nothing new. What is new is the use of this device to monitor uterine activity while the patient is ambulatory and at home. Their results are impressive. I have some questions, however, for Dr. Morrison. The first question is in regards to cost. Does he have any data comparing the cost of using the ambulatory monitor versus the expense eliminated in the intensive care nursery by providing a longer time for the fetus in utero? Table III of the article showed that 23% of patients in the control group had ruptured membranes as compared with 4% in the monitored group. Does he feel that this is attributed to earlier detection of increased uterine activity in the monitored group? Table III also indicates that 67% (22/33) of patients in the control group and 71% (24/34) of patients in the monitored group developed preterm labor. Although these patients were at very high risk, this appears high. Is it a result of overdiagnosis? In Table IV of the article, 10 of 22 patients in the control group were suitable for long-term tocolysis at the time of presentation in preterm labor. This is a high percentage compared with that of most studies where it is approximately 20% to 25%. Is this a result of patient education and the daily telephone calls? Table IV also indicates that 12 of 22 and 13 of 24 patients in the control and monitored groups, respectively, perceived contractions. What does this mean? Studies to date and our own experience would suggest that patients perceive much less of their own uterine activity and the fact that patients do not perceive contractions should not be reassuring. Do Dr. Morrison and his colleagues consider this monitor an effective tool for all preterm labor patients who have risk factors? If not, just how do they decide who should use this device? Newman et al.[1] have also studied home ambulatory monitoring. They observed that normal multifetal gestations that reach term have inherently more uterine activity than a similar singleton gestation. Did Dr. Morrison observe this finding? There is little information on neonatal outcome, and ultimately all of these things are done for neonatal well-being. Could Dr. Morrison indicate whether he saw any improvement in neonatal morbidity in terms of respiratory distress syndrome, birth weight, days on the ventilator, days in the intensive care nursery, intracranial hemorrhage, etc., in the monitored group? This study may be too small to show any change in these data but do the authors have any information for us?

Dr. Morrison and his colleagues are to be congratulated on attempting to define the role of home ambulatory monitoring in the prevention of preterm birth. Although larger numbers and data from other centers will be helpful, studies to date show that home ambulatory monitoring is an effective way to detect preterm labor and prevent preterm delivery by increasing the number of candidates for effective tocolysis.

REFERENCE

1. Newman RB, Gill PJ, Katz M. Uterine activity during pregnancy in ambulatory patients: comparison of singleton and twin gestations. AM J OBSTET GYNECOL 1986;154:530.

DR. EDUARD EICHNER, Cleveland, Ohio. I want to make some comments and commend the group that presented this paper with some data going back some 30 or 40 years ago when we first started monitoring. At that time we would not have been able to use anything like this because our procedure involved placement of electrodes into the myometrium, into the vascular tree, and into the endometrial cavity, so that part of patient cooperation was not necessary.

The other thing is that bed rest for our group with membranes that ruptured after they went home failed and labor started again in almost every patient within a day or two of being at home when she was allowed to be active.

As to the question of prolonged tocolysis, we had 100% success in those days with intravenous Relaxin, but its use was discontinued because it was too costly. Some patients had up to 7 cm of dilatation with the fetus at the +2 station. We would put the bed in a steep Trendelenburg position, and the fetus would go back into the uterine cavity and the cervix would close.

DR. L. RUSSELL MALINAK, Houston, Texas. I would like to ask Dr. Morrison, if the patients were truly ran-

domized, how can he account for the equal distribution of pathologic processes in his report?

Dr. Morrison (Closing). Dr. Mariona asked about the diagnosis of preterm labor and how many visits it took to arrive at this diagnosis. In other words, how many false negative results and how many false positive ones would be the equally important questions.

We had no false negative results. By that I mean that there were no women who had no uterine activity in this relatively small group of patients that were monitored that then came in with significant cervical dilatation. We found that an average of two patient visits was required for the diagnosis of preterm labor. Some of these cases were diagnosed, of course, on the first visit into the obstetric emergency room and some on the third or fourth visit, but about two visits was the average. So we had about a 20% rate of false positive diagnoses where the patients would have contractions and then would come in at some time and be told, "No, you are not in preterm labor." That includes the ones that went into labor and the ones that did not.

I think we cannot overemphasize Dr. Mariona's point about a cervicovaginal examination and its contribution to the prevention of preterm birth.

It is well known that the flora of the vagina, if one strips the membranes and invades the cervix, can actually cause labor. I do not have to tell many of you in this room about that system, which does work. So what we are talking about here is simply an assessment of the lower uterine segment and in general how effaced and dilated the cervix is without invading the cervix. I think that needs to be underscored.

Of course we did not do any cervical flora analysis in this study.

The other question related to why three of 10 women, who essentially were good candidates for tocolysis in the palpation group, went into preterm labor, had labor stopped, but were delivered before term. Two of those three women came back at 7 and 10 days after discharge on a regimen of oral tocolytics and experienced resumption of preterm labor. Of course they were assessing their contractions by palpation and on readmission were not good candidates for tocolysis. In other words, the cervix was more significantly dilated. Although they were again treated with intravenous tocolytics, they were delivered within a week of the second treatment.

Intercourse and bed rest were mentioned. Was intercourse interdicted after the patients went into preterm labor? The answer is yes, but we individualized that variable. In other words, if they were at home for 2 or 3 weeks and had no recurrence of contractions, then we gradually allowed ambulation and eventually intercourse. The patients were allowed to resume fairly normal activity if they were not having excess uterine activity while on a regimen of tocolytics.

Dr. Hill mentioned two interesting points. One was about expense and the other was about significant neonatal complications. Both the first and last point he derived were, I think, two of the more important points mentioned.

The expense data and the neonatal complication data will be presented at a separate time, but basically it was much more expensive to have those babies in the neonatal intensive care unit for a longer period of time. They were not being kept just for the good of the mother or anyone else. They had significant complications such as respiratory distress syndrome and necrotizing enterocolitis. In the monitored group the babies were mostly delivered at term and went to the normal newborn nursery, so they did not have many of those significant complications. Therefore we think that both expense and complications are significantly reduced.

Dr. Hill mentioned that 38% were good candidates for tocolysis in the control group, and he felt this was a relatively high figure. I think it depends on which literature you read. Some of the literature, such as the data of Papiernik et al.[1] from France, would indicate there could be even a higher percentage than that. I think that this reflects the intensive education and close follow-up by pelvic examinations these patients were given, and that is why they actually presented that often as good candidates for tocolysis.

Clearly only the patients with risk factors for preterm labor should be monitored. I think the greatest danger of this technique, like most new technologies, is that it will be over-utilized or utilized in the wrong group of patients.

There is no question in my mind that if this device is used for those who do not have high-risk factors for preterm labor, some of the "soft" indications, if you will, then it is not going to be cost effective or health effective. The message that I would leave you with on that score is that you must make sure that the patient is at high risk for preterm delivery before prescribing this device or it will be inadequately used.

I think this reflects, since we required two or more previous preterm deliveries in our group of patients, why our rates of detection of preterm labor were so high, 71% and 67%, respectively.

Also I would like to underscore Dr. Hill's comments on maternal perception of uterine activity. We did not mean to suggest that the fact that patients could feel 50% of their contractions was indicative that this was a good method of detection of uterine activity leading to preterm labor. We meant it the other way around, that is, that 50% of the patients could not significantly detect preterm labor. Half of a patient population or, by the study of Newman et al.,[2] approximately 80% of those in true preterm labor will be missed on accurate palpation to determine uterine activity that would in fact lead to preterm labor. So we do not think these signs or the percentages of women who perceive contractions are reassuring.

We agree with Newman et al.[3] that twins are associated with more uterine activity. In the patients with twin gestations we individualized the uterine activity

monitor. In other words, twins produced more activity, and sometimes we would have to raise the baseline from three contractions to five or six contractions per hour. If patients came in with four contractions an hour and did not have any cervical change in two or three assessments, we would simply raise the baseline because even twins who are delivered at term will produce more uterine activity than singleton gestations.

Dr. Eichner raised the possibility of Relaxin, and it is possibly going to enjoy a comeback. A group in St. Louis and a group in, I think, Minnesota are working with that compound now, so we may see it on the horizon as a therapeutic agent for those in preterm labor.

Finally Dr. Malinak raised the question of randomization. I really cannot say why the distribution was so equal except to say that we accepted patients on a first-come, first-served basis and randomized them. That is the way the data turned out.

We particularly looked at the six patients that refused randomization, and they were similar to our study population. I thought perhaps some of those might have skewed the data. We did have a few more twins in the monitored group when compared with the palpation group, so the groups were approximately but not exactly equal.

REFERENCES

1. Papiernik E, Bouyer J, Collin D, Winisdoerffer G, Dreyfus J. Precocious cervical ripening and preterm labor. Obstet Gynecol 1986;67:238.
2. Newman RB, Gill PJ, Wittreich P, Katz M. Maternal perception of prelabor uterine activity. Obstet Gynecol 1986; 68:765.
3. Newman RB, Gill PJ, Katz M. Uterine activity during pregnancy in ambulatory patients: comparison of singleton and twin gestations. AM J OBSTET GYNECOL 1986;154:530.

Braxton Hicks' contractions and motor behavior in the near-term human fetus

E. J. H. Mulder, M.Sc., and G. H. A. Visser, M.D., Ph.D.
Groningen, The Netherlands

The transient relationship between Braxton Hicks' contractions and fetal motor behavior was studied in 14 healthy nulliparous women near term. Two-hour recordings of fetal heart rate and uterine contractions and of real-time scanning for fetal body movements, breathing, and eye movements were made. The recordings were divided into state 1F and non–state 1F. Braxton Hicks' contractions were not influenced by fetal behavioral states and state changes were not related to these contractions. Fetal body movements did not stimulate contractions, but contractions coincided with a specific clustering of body movements during the ascending part of contractions. Breathing was clustered during the descending part of short-lasting contractions but decreased gradually during the long-lasting ones. Heart rate variation was increased during contractions. (AM J OBSTET GYNECOL 1987;156:543-9.)

Key words: Braxton Hicks' contractions; fetal behavioral states, fetal body movements, fetal breathing movements, fetal heart rate accelerations

More than a hundred years ago, J. Braxton Hicks[1] described the existence of periods of spontaneous antepartum uterine activity in the human. During late pregnancy the rhythmicity of these so-called Braxton Hicks' contractions constitutes a remarkably regular feature of the fetal environment in every pregnant woman. Clinically, these contractions have become important for fetal heart rate monitoring, and presence or absence of late decelerations after Braxton Hicks' contractions is considered to be an important indicator of the fetal condition. Little is known, however, as to the reaction of the normal fetus to these contractions or if the fetus itself influences the recurrence rate of these contractions by its movements.

In sheep, fetal behavioral states may be influenced by prolonged low-amplitude prelabor uterine contractions ("contractures"), often changing rapid eye movement sleep to a sleep state in which eye movements are

From the Department of Obstetrics and Gynaecology, University Hospital.
Received for publication May 6, 1986; revised September 22, 1986; accepted September 26, 1986.
Reprint requests: E. J. H. Mulder, Department of Obstetrics and Gynaecology, University Hospital, Oostersingel 59, 9713 EZ, Groningen, The Netherlands.

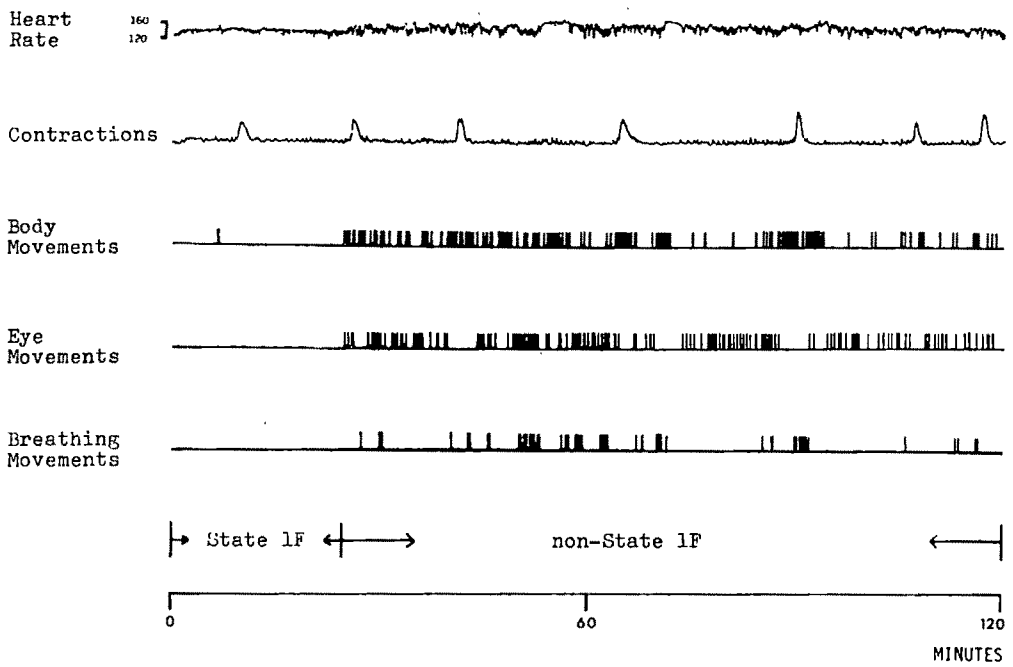


Fig. 1. Example of a 2-hour recording, showing, from above downward, the fetal heart rate tracing and the occurrence of uterine contractions and body, eye, and breathing movements. The presence of the two distinct behavioral states is indicated.

absent.[2] These contractures were not initiated or modified by fetal body movements.[3]

The near-term human fetus exhibits four distinct behavioral states,[4] which resemble those found in the neonate.[5] Because it is possible that in the human these frequently occurring periods of myometrial activity influence fetal behavior, we studied the temporal relationship between Braxton Hicks' contractions and motor behavior in the near-term human fetus, taking into account the fetal behavioral states. In this study we also investigated the role of fetal body movements as a possible controlling factor of myometrial activity.

Subjects and methods

Fourteen healthy nulliparous women were invited to participate in a study that combined real-time ultrasound scanning, monitoring of fetal heart rate (FHR), and recording of uterine activity. All women were outpatients with normal singleton pregnancies of 38 to 40 weeks' gestation. Subsequent delivery and outcome were uneventful; all infants were appropriate for dates (birth weight 3501 ± 465 gm, mean ± SD) and did well at and after birth.

Continuous on-line recordings lasting ≥2 hours were made between 3:00 and 6:00 PM with the women in a semirecumbent position, slightly tilted to the left. Fetal pulse (R-R) intervals were derived from abdominal electrocardiographic electrodes; they were recorded on a cardiotocograph strip chart (Hewlett Packard Model 8030 A) as beats per minute and, after having been preprocessed by means of a microprocessor, they were stored on a minicassette. These data were analyzed with the use of a computer program, of which a detailed description has been published,[6] yielding information on variations around the basal heart rate (accelerations and decelerations, expressed as the root mean square value). FHR was also recorded on an event recorder (Hewlett Packard Model 7754 A) at a paper speed of 1 mm/sec and stored on a magnetic-tape recorder (Philips, Analog-7).

Records of uterine activity were obtained with the external tocotransducer of the same cardiotocograph and written on its strip chart. The tocographic signal was, after amplification, also recorded on the event recorder and stored on magnetic tape.

Fetal activity was identified by means of two linear-array real-time scanners (Model SSD-256, Aloka Co., Tokyo, Japan, and Imager 2300, Siemens AG, Erlangen, Federal Republic of Germany). Both probes were 3.5 MHz. One observer recorded fetal eye movements; another one, fetal body and breathing movements. All types of fetal movements were recorded by means of handheld pushbuttons on the event recorder, together with the FHR and tocographic signal, and were also stored on magnetic tape. An example of a compressed 2-hour record is shown in Fig. 1. Body movements (excluding isolated limb movements, startles, and isolated movements of the head) occurring within 5 sec-

Table I. Overall incidence of uterine contractions, state 1F, and fetal body and breathing movements per fetus

Case No.	Recording time (min)	Contractions				State 1F	Body movements	Breathing movements
		No./hr	Duration (sec) Mean ± SEM	Duration (sec) Range	% of recording time			
1	60	23.0	70.2 ± 6.7	45-163	52.8	25.2	10.9	11.0
2	158	14.1	105.5 ± 9.2	46-278	41.2	13.3	15.7	26.6
3	137	5.7	147.7 ± 23.0	75-409	23.3	31.5	21.9	2.3
4	120	16.5	119.1 ± 7.2	68-258	54.0	17.8	16.8	0.2
5	128	4.2	201.0 ± 70.4	66-728	23.5	31.1	13.7	18.5
6	120	22.0	85.1 ± 4.2	45-161	51.5	27.9	7.3	0
7	134	17.0	97.1 ± 7.0	46-236	45.9	16.0	26.6	6.6
8	120	4.0	162.8 ± 26.7	68-328	18.1	15.8	10.8	16.2
9	124	4.4	223.6 ± 75.8	58-840	28.5	10.6	15.6	34.4
10	120	3.0	130.8 ± 19.4	93-233	10.9	21.8	15.9	5.5
11	130	2.8	290.5 ± 28.9	194-395	22.3	10.8	29.3	30.3
12	120	16.5	98.2 ± 9.6	45-332	46.3	17.9	14.0	15.6
13	60	15.0	97.9 ± 17.3	46-334	40.9	38.9	4.7	2.1
14	140	8.6	116.8 ± 10.8	45-281	27.7	20.0	31.0	52.3
Mean	119	11.2	113.8		34.8	21.3	16.7	15.8
SEM	7	1.9	4.8		3.7	2.2	2.0	4.2

If not stated otherwise the incidence is expressed as the percentage of recording time.

onds of each other were considered to be a single movement. Their incidence was expressed as a percentage of recording time. For breathing movements apnea was defined as a pause between two breaths of ≥6 seconds. The incidence was expressed as a percentage of recording time or as the number of breaths per time unit.

Fetal behavioral states, states 1F through 4F (the suffix F indicates fetal), were identified according to the criteria of Nijhuis et al.[4] However, since states 3F and 4F are scarce, we divided the recordings only into state 1F ("quiet" sleep: stable FHR with low heart rate variability, body movements absent or incidental, and eye movements absent) and non–state 1F.

External tocography provided information on the approximate duration of the uterine contractions. Only contractions lasting ≥45 seconds were included (n = 294). These represented 81% of all observed contractions. The incidence of contractions was expressed as the percentage of recording time (total or per sleep state).

Statistical analysis was performed with the Wilcoxon signed-rank test, unless stated otherwise. The significance levels given are for two-sided tests.

Results

A total of 29 hours of observation was obtained from the 14 women. One hour (3.5% of total recording time) had to be excluded from the analysis of contractions because of incorrect placing of the tocotransducer. For two cases detailed analysis of FHR could not be performed because of poor quality of the signal (failure time >50%).

Details on the incidence of contractions, fetal movements, and occurrence of state 1F in the individual cases are shown in Table I. On average 11 contractions occurred per hour (35% of total recording time). The mean duration of the contractions was 114 seconds; 71% lasted <120 seconds; 25% from 120 to 300 seconds; and nearly 4% >300 seconds (Fig. 2).

All 14 fetuses showed at least one episode of state 1F. In total 19 of these episodes were observed, of which 16 were enclosed periods (mean duration 17.6 minutes, range 6 to 26 minutes). The fetuses spent on average 21% of recording time in state 1F. The mean incidences of body and breathing movements were 16.7% and 16%, respectively. During non–state 1F the mean duration of a single bout of body movements was 13.0 seconds (range of means among the individual fetuses from 4.4 to 24.0 seconds).

For none of the three parameters taken for comparison was a significant difference found in the incidence of uterine activity between state 1F and non–state 1F (Table II).

During the 5 minutes preceding and following each change into and/or out of state 1F, the percentage of contraction time was calculated. The mean incidence of uterine contractions in each of the four distinct 5-minute periods did not deviate largely from the mean incidence observed for the entire recording time (~35%, Table I). Neither for the changes from state 1F to non–state 1F (n = 19) nor for the changes from non–state 1F to state 1F (n = 16) was the incidence of contractions before the state change found to be significantly different from that after the change; the mean values were 33% as compared with 41% and 39% as compared with 34% of time, respectively. Moreover,

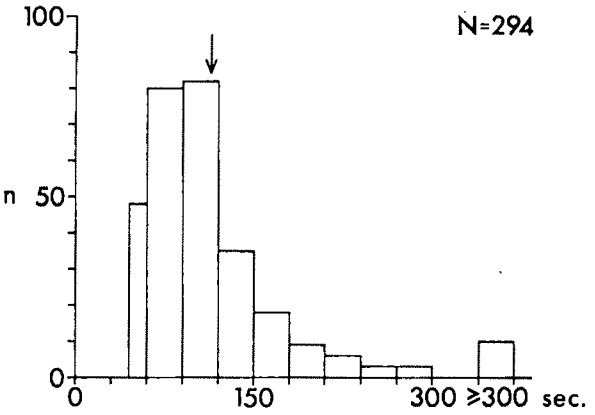


Fig. 2. Distribution of the duration of 294 uterine contractions, lasting ≥45 seconds. The mean duration (114 seconds) is indicated by the *arrow*.

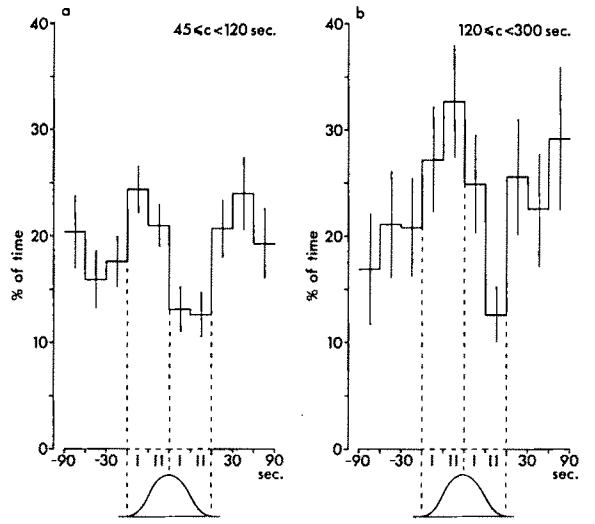


Fig. 3. Incidence of body movements before, during, and after *(a)* short-lasting (n = 123) and *(b)* long-lasting (n = 37) Braxton Hicks' contractions, occurring during non–state 1F (mean ± SEM). Both the ascending and descending parts of the contractions were subdivided into two sections of equal duration.

the incidence of long-lasting Braxton Hicks' contractions (duration between 120 and 300 seconds) and prolonged contractions (lasting more than 300 seconds) was not increased around the time of these state changes. However, the longest contraction we observed (840 seconds) was associated with a FHR deceleration, and during this contraction a change from non–state 1F to state 1F occurred.

The incidence of body and breathing movements during contractions did not differ significantly from that found between the contractions; this held both for state 1F and for non–state 1F (Table III). During the contractions, however, a specific patterning of body and breathing movements was found (Figs. 3 and 4). Body

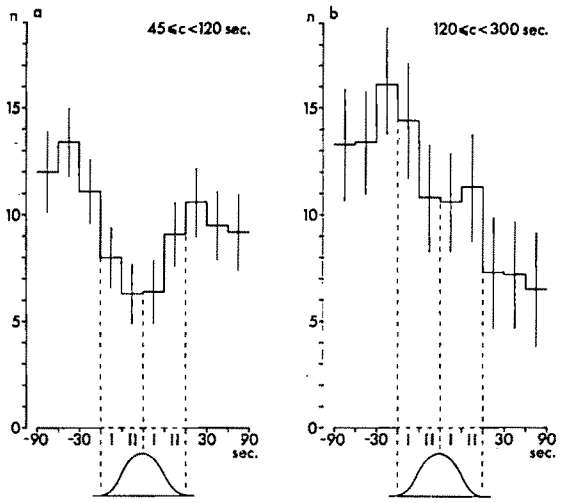


Fig. 4. Incidence of breathing movements (number per 30 seconds) before, during, and after *(a)* short-lasting (n = 53) and *(b)* long-lasting (n = 23) Braxton Hicks' contractions (mean ± SEM).

Table II. Incidence of uterine contractions during state 1F and non–state 1F

Parameter	*State 1F*	*Non–state 1F*
No. of contractions/hr (mean ± SEM)	9.7 ± 1.9	11.3 ± 1.8
% of contraction time (mean ± SEM)	31.2 ± 5.6	33.1 ± 4.2
Duration of contractions (mean ± SEM)	114.7 ± 9.2	107.6 ± 6.3

None of the differences was statistically significant.

movements showed a clustering in the ascending part of the contraction, followed by a significant decrease during the descending part ($p < 0.01$, as compared with the ascending part). This distribution was found for both categories of contractions (Fig. 3, *a* and *b*), but the increase during the ascending part was only significant for the long-lasting Braxton Hicks' contractions ($p < 0.02$, as compared with the control period).

The number of breathing movements per 30-second epoch decreased significantly ($p < 0.05$) during the ascending part of the short-lasting Braxton Hicks' contractions but increased again as uterine activity decreased (Fig. 4, *a*). For the long-lasting contractions such a troughlike pattern was not found, but instead there was a pattern with a more or less gradual decrease in the course of the contraction, which continued thereafter (Fig. 4, *b*).

For this detailed analysis the incidence of body movements in the course of the contractions was studied only during non–state 1F ("active" sleep), whereas breathing movements were studied if they occurred during a contraction or in the 90 seconds preceding or following

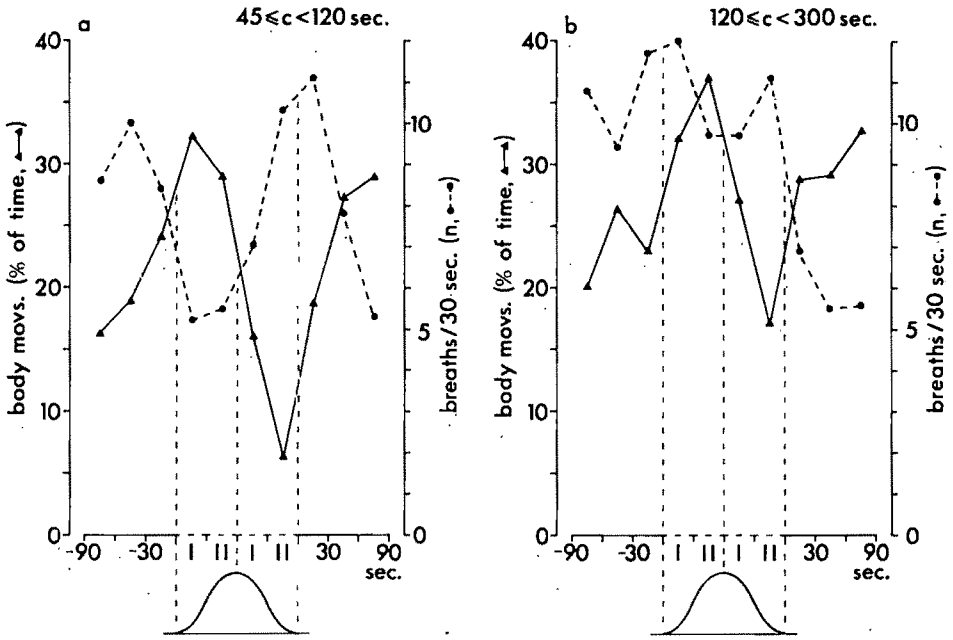


Fig. 5. Body movements and breathing movements before, during, and after *(a)* short-lasting (n = 36) and *(b)* long-lasting (n = 19) Braxton Hicks' contractions. Selected were those contractions, occurring during non–state 1F, where in the period of analysis both body and breathing movements occurred.

Table III. Incidence of body and breathing movements during and in the absence of contractions, for both behavioral states

	State 1F (% of time, mean ± SEM)		*Non–state 1F (% of time, mean ± SEM)*	
	During contractions	*Between contractions*	*During contractions*	*Between contractions*
Body movements	1.1 ± 0.6	1.3 ± 0.6	19.8 ± 2.8	19.3 ± 2.5
Breathing movements	11.9 ± 4.2	16.3 ± 5.0	16.6 ± 4.3	15.2 ± 4.0

that contraction. For those contractions that met both criteria (n = 55), a specific distribution of these movements was found (Fig. 5). An inverse relationship between the clusters of body and breathing movements was demonstrated for the short-lasting contractions (Fig. 5, *a*). The distribution patterns of body and breathing movements in the course of the long-lasting contractions (Fig. 5, *b*) were akin to those presented in Figs. 3, *b*, and 4, *b*, respectively.

Fetal heart rate accelerations (≥15 bpm; ≥15 seconds) occurred almost exclusively during non–state 1F. In the 12 fetuses in which this was studied only 2% of the accelerations occurred during state 1F. Ninety-seven of the 331 accelerations observed during non–state 1F (29.3%) started during a contraction. This approximates the percentage of time during which contractions are present in non–state 1F (33.1%, Table II); accelerations are therefore not induced by contractions. The mean duration of the accelerations that occurred during a contraction was, however, significantly longer than that of accelerations not associated with contractions (58.2 ± 3.4 and 50.3 ± 3.1 seconds, respectively, mean ± SEM; $p < 0.01$). Heart rate variation during contractions and in the first minute thereafter was significantly higher ($p < 0.05$) than during the 3 minutes preceding the contractions (Fig. 6). The percentage of accelerations that were associated with body movements was equally high during and between contractions (92.4 and 93.1, respectively; binomial test NS).

Comment

In the human, periods of antepartum myometrial activity were described by J. Braxton Hicks[1] as early as 1872. He was convinced of "the fact that the uterus possesses the power and habit of spontaneously contracting and relaxing from a very early period of preg-

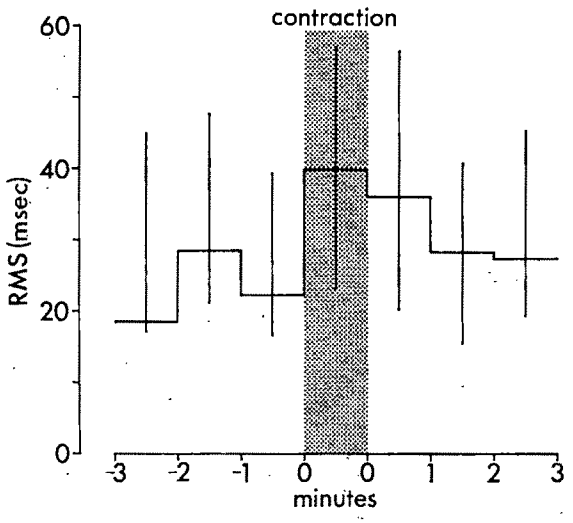


Fig. 6. Heart rate variation, expressed as the root mean square *(RMS)* value, before, during, and after short-lasting Braxton Hicks' contractions (n = 31), occurring during non–state 1F. Only those contractions that were not preceded or followed by another contraction within 3 minutes were selected for this analysis. Given are the medians and interquartile ranges.

nancy (third month)." He detected the contractions manually through the vagina and observed that they occurred every 5 or 10 minutes, sometimes even twice in 5 minutes. The duration of each contraction was generally not long; ordinarily each lasted from 2 to 5 minutes.[1]

The occurrence of antepartum uterine contractions was confirmed by Caldeyro-Barcia and Poseiro,[7] who studied intrauterine pressure by means of abdominally inserted open-tip catheters, in more than 500 women. On average, two to three contractions appeared to occur per 10-minute interval near term. This corresponds well with the incidence of nearly two per 10 minutes as found with external tocography in the present study. When placed correctly, the transducers detect contractions fairly reliably, although no information is obtained on their intensity.[8] At present, little information is available about the influences of Braxton Hicks' contractions on fetal behavior or vice versa.

In the human fetus behavioral states develop between 36 and 38 weeks of gestation. These states are distinct and mutually exclusive modes of neural activity, reflected by clusters of phenomena, which are stable over time and recur repeatedly.[5]

The period of state 1F, which is defined as a period without or almost without fetal body movements, provides us with an "in-built" control period, as we can compare periods with and without body movements in relationship to contractions in the same individual. Consequently the possible role of fetal body movements in the initiation of uterine contractions, for instance, by physical stimulation or irritation of the myometrium, could be analyzed by this study design. In turn, the possible influences of contractions on fetal activity and fetal behavioral states could also be investigated.

During state 1F the incidence of uterine contractions was the same as that during non–state 1F. This means that the occurrence of contractions (frequency, duration) is not influenced by the presence of either behavioral state. Since state 1F includes no or very few body movements, this implies also that it is very unlikely that fetal movements induce or modify contractions.

The present study shows that in the human, as in the sheep,[1, 2] prolonged contractions (lasting more than 5 minutes) do occur. On average one per 2 to 3 hours was observed. However, we could not find any influence of these prolonged contractions or of Braxton Hicks' contractions on behavioral states, as their incidences were not altered near state changes. Prolonged contractions associated with heart rate decelerations, however, change the fetal heart rate pattern from high to low variability, and in the few cases in which eye and body movements were observed, these movements also appeared to arrest temporarily.[9] Consequently it seems, in the human, that only in situations of supposed fetal hypoxemia do prolonged contractions change fetal behavioral states from rapid eye movement sleep to non–rapid eye movement sleep.

In this study we found that in both sleep states the overall incidence of body and breathing movements during contractions was the same as in between contractions. This indicates that contractions do not alter the overall incidence of these movements. There was, however, a specific patterning of both types of movements during the contractions. The decline of breathing in the first half of the contraction, followed by an increase during the descending part, was also found by Wilkinson and Robinson.[10] They suggested that this patterning might be due to distortion of the fetus, changing sensory input from chest wall and lung afferents, as was also reported in sheep.[2] On the other hand, it might well be a consequence of the patterning of body movements, as it is known that fetal breathing decreases during body movements.[11, 12] The gradual decline of breathing, which continued after the long-lasting contractions, might be related to a decreased fetoplacental exchange. The increase of body movements during the ascending part of contractions suggests a stimulation of these movements by contractions. That this does not occur during state 1F ("quiet" sleep) is in accordance with an earlier study that showed that neither body movements nor heart rate accelerations are induced by external physical stimulation of the fetus through the maternal abdominal wall[13] and with the fact that neonates are difficult to arouse during quiet sleep. In the fetus bursts of movements occur at more or less regular intervals of about 1 minute.[14] It is there-

fore understandable that an increase of movements during the first half of the contraction is followed by a decrease afterward, resulting in an equal overall incidence of body movements during and between contractions.

The relationship between heart rate accelerations and body movements found in this study, is in agreement with reports in the literature.[11, 15] Heart rate accelerations are often followed by transient decelerations.[11] The patterning of body movements during the contractions might therefore explain the transient increase in heart rate variation. The longer duration of the accelerations during contractions suggests, however, that other mechanisms might also play a role. One may think in terms of qualitative changes in body movements (more forceful) or of compensatory heart rate changes induced by the transient reduction in fetoplacental exchange during the contractions.

It is concluded that in the near-term human fetus: (1) Braxton Hicks' contractions are not influenced by behavioral states, and state changes are not related to these contractions; (2) body movements do not stimulate contractions; (3) contractions coincide with a specific clustering of body movements and breathing movements.

We thank K. T. Meijer for his expert technical help. Our thanks are also due to Dr. A. Boogert for the critical reading of the manuscript and to Y. Lems for the illustrations.

REFERENCES

1. Braxton Hicks J. On the contractions of the uterus throughout pregnancy: their physiological effects and their value in the diagnosis of pregnancy. Trans Obstet Soc Lond 1872;13:216-31.
2. Nathanielsz PW, Bailey A, Poore ER, Thorburn GD, Harding R. The relationship between myometrial activity and sleep state and breathing in fetal sheep throughout the last third of gestation. Am J Obstet Gynecol 1980; 138:653-9.
3. Nathanielsz PW, Yu HK, Cabalum TC. Effect of abolition of fetal movement on fetal intravascular Po_2 and incidence of tonic myometrial contractures in the pregnant ewe at 114 to 134 days' gestation. Am J Obstet Gynecol 1982;144:614-8.
4. Nijhuis JG, Prechtl HFR, Martin CB Jr, Bots RSGM. Are there behavioural states in the human fetus? Early Hum Dev 1982;6:177-95.
5. Prechtl HFR. The behavioural states of the newborn infant (a review). Brain Res 1974;76:185-212.
6. Dawes GS, Visser GHA, Goodman JDS, Redman CWG. Numerical analysis of the human fetal heart rate: the quality of ultrasound records. Am J Obstet Gynecol 1981; 141:43-52.
7. Caldeyro-Barcia R, Poseiro JJ. Oxytocin and contractility of the pregnant human fetus. Ann NY Acad Sci 1959; 75:813-30.
8. Nagel J, Schaldach M. The non-invasive assessment of uterine activity. In: Rolfe P, ed. Non-invasive measurements. ed 2. London: Academic Press 1983:105.
9. Visser GHA, Heringa MP, Mulder EJH, Bekedam DJ. Effects of uterine contractions, associated with fetal heart rate decelerations, on human fetal behavioural states. In: Fetal and neonatal physiological measurements, second international conference, April 1984. Oxford: Express Litho Service, 1984:41-2.
10. Wilkinson C, Robinson J. Braxton Hicks' contractions and fetal breathing movements. Aust NZ J Obstet Gynaecol 1982;22:212-4.
11. Dawes GS, Visser GHA, Goodman JDS, Levine DH. Numerical analysis of the human fetal heart rate: modulation by breathing and movement. Am J Obstet Gynecol 1981;140:535-44.
12. Roberts AB, Little D, Cooper D, Campbell S. Normal patterns of fetal activity in the third trimester. Br J Obstet Gynaecol 1979;86:4-9.
13. Visser GHA, Zeelenberg HJ, de Vries JIP, Dawes GS. External physical stimulation of the human fetus during episodes of low heart rate variation. Am J Obstet Gynecol 1983;145:579-84.
14. Robertson SS, Dierker LJ, Sorokin Y, Rosen MG. Human fetal movement: spontaneous oscillations near one cycle per minute. Science 1982;218:1327-30.
15. Sadovsky E, Rabinowitz R, Freeman A, Yarkoni S. The relationship between fetal heart rate accelerations, fetal movements, and uterine contractions. Am J Obstet Gynecol 1984;149:187-9.

(Sub)Specialization in obstetrics and gynecology: Results of a survey by The American Board of Obstetrics and Gynecology

James A. Merrill, M.D.
Seattle, Washington

Survey of department chairmen (n = 113) and former fellows (n = 599) revealed that from 1974 to 1984 the mean number of full-time faculty doubled and the increase was in subspecialists (5.1 per department). More than 60% of departments report trained subspecialists in three areas and 92.9% in one or more areas. Twenty-two chairmen report zero laboratory research funding. Of former fellows, 82% are certified in obstetrics and gynecology; 44% are certified in a subspecialty; 86.3% have passed the subspecialty written examination. Employment is full-time faculty for 60.8% and full-time private practice for 16.1%. They devote 49.1% of professional time to the subspecialty and 15.9% to research, most of which is clinical; 56.6% spend no time in laboratory research. While 40.3% have submitted no research grant applications since completion of fellowship, of all grants submitted to the federal government, 63.5% were approved and 34.5% were funded. The mean number of papers published since fellowship is 7.8. The number of fellows graduating may meet estimated need. The initial objectives of this new certification are being achieved. (AM J OBSTET GYNECOL 1987;156:550-7.)

Key words: Subspecialization, specialization, obstetrics and gynecology, survey

The American Board of Obstetrics and Gynecology was incorporated in 1930 and the first specialists in obstetrics and gynecology certified. The board continues to be interested in the nature of physicians who enter obstetrics and gynecology, the quality of education they receive, and the impact of the evaluation processes of the board. In 1969 the board convened a conference on specialization, attended by representatives of national societies in obstetrics and gynecology. The purpose of the conference was to improve the health care of women by elevating standards of education. The conference was followed by thorough study of the impact of advanced training in obstetrics and gynecology. An outgrowth of this was the formation, in 1972, of three specialty divisions of The American Board of Obstetrics and Gynecology for (1) gynecologic oncology, (2) reproductive endocrinology, and (3) maternal-fetal medicine.

The general objective of the specialty divisions was to improve the health care of women with special problems by: (1) elevating standards of education, (2) enhancing the recruitment of qualified physicians, (3) improving the organization and distribution of patient care, and (4) increasing basic knowledge. Although not specifically stated, many assumed that "increasing basic knowledge" would result from increased research activities of specialists certified for special qualification.

Criteria and mechanisms were established for approval of specialty fellowships. Each is periodically reviewed by the Board. By 1985 there were 161 approved fellowship programs (29 in gynecologic oncology, 50 in reproductive endocrinology, and 82 in maternal-fetal medicine). In 1974 the first examinations were given and certificates of special qualification were issued. By the end of 1985 there were 896 diplomates who had been awarded certificates of special qualification in one of the three specialty areas (364 in maternal-fetal medicine, 294 in gynecologic oncology, and 238 in reproductive endocrinology).

At 10 years after the first certificates of special qualification were granted, the Board was interested in studying the results of this new specialty certification. Many of the original objectives seemed to have been accomplished. However, concern had been expressed that research in obstetrics and gynecology may have been diminished by substitution of clinically oriented specialty training programs for the laboratory training that otherwise might have been pursued by those entering academic careers. Therefore three studies were conducted by the board addressing these concerns. One was of the scholastic ability of residents in obstetrics and gynecology completing their training in 1983 and 1984. Reported here are analyses of a questionnaire sent to all who had ever been enrolled in a board-approved specialty fellowship and a questionnaire sent to all chairmen of medical school departments of obstetrics and gynecology.

From The American Board of Obstetrics and Gynecology, Inc.
Received for publication August 12, 1986; accepted September 26, 1986.
Reprint requests: James A. Merrill, M.D., The American Board of Obstetrics and Gynecology, Inc., 4507 University Way N.E., #204, Seattle, WA 98105.

Method

In 1984 the two questionnaires were sent out. A questionnaire designed to obtain information regarding laboratory research activity in medical school departments of obstetrics and gynecology and the impact of specialty fellowship training was sent to 144 chairmen of departments of obstetrics and gynecology in the United States and Canada. The second questionnaire designed to identify activity profile, with emphasis on laboratory research, was sent to the 869 individuals who had started a Board-approved fellowship prior to 1982, thus to those presumably who had completed fellowship prior to 1984. (In fact, seven respondents indicated they started their fellowship in 1982 and two respondents indicated that they started in 1983.) The data were compiled, analyzed, and made available by the Division of Research in Medical Education, University of Washington, School of Medicine, Seattle, Washington.

Response

The response was good. The questionnaire was completed and returned by 116 department chairmen (80.6%). The questionnaires from 113 chairmen (78.5%) including 108 from the United States were used in the analysis. Questionnaires were received from 614 former fellows (70.7%). The responses from 599 (69.2%) former fellows were used in the analysis. The only questionnaires excluded from the analyses were those received after computations had been initiated. There were a few questions with no responses in one or more instances and some completed the questionnaire but did not identify their specialty area.

Findings and results

Chairman questionnaire. Between 1974 and 1984 the number of full-time faculty in departments of obstetrics and gynecology nearly doubled (Table I). Although 17.9% of departments in 1974 had no M.D. full-time faculty, in 1984 the mean number per department was 12.6. The increase in full-time faculty has resulted from appointment of individuals with special qualification in the specialty areas of obstetrics and gynecology (Table II). The mean number of trained subspecialists per department is: reproductive endocrinology, 1.4; gynecologic/oncology, 1.4; maternal-fetal medicine, 2.3. There are about half again as many individuals in departments with subspecialty interest who have not completed approved specialty fellowships. Table III shows that only 7% of departments do not have any faculty member who has completed a specialty fellowship. More than 60% of departments report fellowship-trained specialists in all three areas; 23.9% in two areas; and almost 9% in one area only. The proportion and number of fellowship-trained specialists in medical school departments of obstetrics and gynecology are increasing.

Table I. Number of full-time faculty (mean per department)

	M.D.	*M.D. and Ph.D.*	*Ph.D.*
In 1974	6.6*	0.4	1.1
In 1984	12.6	0.7	2.3

*17.9% of departments had none.

Analysis of the questionnaire suggests that laboratory research activity in departments of obstetrics and gynecology is not flourishing. Almost one third of chairmen (29%) indicated they could not allocate research laboratory space to a new faculty member and 40.4% of the chairmen indicated that no one had been trained in laboratory research in the department during the previous 10 years. As expected, laboratory research support, in order of dollar amount, decreases from federal government to foundations, practice revenues, industry, and university. For each of these categories of funding, more than 25% of the schools indicate no laboratory research funding. There were 22 department chairmen who reported zero laboratory research funding from all sources combined. The chairmen were asked if they plan, within the next 5 years, to recruit new faculty who will devote more than 50% of their time to laboratory research. Table IV demonstrates that 53.6% of the responding chairmen do not plan to recruit an M.D. to do laboratory research. The data indicate that Ph.D.'s are being recruited for research more often than M.D.'s. The mean number of new faculty being recruited for research in the next 10 years is two per school.

The response from chairmen suggests satisfaction with the current number of trainees in obstetrics and gynecology. Table V indicates that slightly more than half of the chairmen recommend that the number of trainees in fellowships and residencies remain stable. Of the respondents, 32.5% recommend an increase in maternal-fetal medicine fellows and 41.5% recommend a decrease in the number of residents.

The chairmen indicated their opinion that the most frequent factors with negative impact on a young faculty member pursuing a career in laboratory research were personal preference and volume of clinical practice.

Fellows questionnaire. Table VI records the specialty area of the 599 respondents whose data were used in the analysis. All of the respondents began a fellowship, but not all of them completed it. Table VII, which displays the date training was completed, shows the growth of fellowship programs, which began in 1973 and increased erratically through the years covered by

Table II. Number of full-time faculty in each subspecialty (mean per department)

	Maternal-fetal medicine	*Gynecologic oncology*	*Reproductive endocrinology*	*Other*
No fellowship	1.1	0.6	0.7	1.0
Complete fellowship	2.3	1.4	1.4	0.2
Total	3.4	1.9	2.2	1.2

Table III. Number of departments with full-time faculty who completed approved fellowships

	n	%
None	8	7.1
All three	68	60.2
Maternal-fetal medicine and gynecologic oncology	12	10.6
Maternal-fetal medicine and reproductive endocrinology	8	7.1
Gynecologic oncology and reproductive endocrinology	7	6.2
Maternal-fetal medicine only	7	6.2
Gynecologic oncology only	2	1.8
Reproductive endocrinology only	1	0.9

Table IV. Recruitment plans: "In the next 5 years how many new faculty do you plan to recruit who will devote more than 50% of their time to laboratory research?"

Faculty degree	*Mean*	*Percent of schools recruiting none*
M.D.	0.7	53.6
Ph.D.	1.0	39.6
M.D. and Ph.D.	0.3	80.7

Table V. Recommended change in the total number of fellows and residents (percent of chairmen)

	Increase (%)	*Decrease (%)*	*Remain stable (%)*
Maternal-fetal medicine	32.4	13.9	53.7
Gynecologic oncology	18.3	23.9	57.8
Reproductive endocrinology	23.1	19.4	57.4
Residents	13.2	41.5	45.3

Nonrespondents were maternal-fetal medicine, five; gynecologic oncology, four; reproductive endocrinology, five; residents, seven.

the survey. There has been no increase in number of programs or number of fellows in recent years. On the basis of the number of fellows who have recently graduated and those currently enrolled in fellowship there appears to be a plateau in the number of physicians completing fellowship at the current rate of 12.8% of the annual graduates from obstetrics and gynecology residencies.

Table VI. Specialty area of 599 respondents

	n	%
Maternal-fetal medicine	245	41.4
Reproductive endocrinology	192	32.4
Gynecologic oncology	155	26.2
Not recorded	7	

Table VII. Date fellowship training completed

	n	%
1970	1	0.2
1974	9	1.6
1975	18	3.2
1976	34	6.1
1977	37	6.6
1978	56	10.0
1979	54	9.7
1980	86	15.4
1981	91	16.3
1982	72	12.9
1983	95	17.0
1984	6	1.1
Nonrespondents	41	

Table VIII reveals that 82% of those responding to the question were certified diplomates in obstetrics and gynecology with almost 85% of gynecologic oncologists certified. If it is assumed that those who did not respond to this question are not certified, then 79.6 of the total respondents are certified. The percent certified in obstetrics and gynecology is no less than the number estimated to be eligible for certification, on the basis of the dates when fellowship was completed. In fact, only 76.3% of respondents indicated that they completed fellowship prior to 1983. Certainly some of those who did not complete fellowship have gone on to be certified in obstetrics and gynecology.

Tables IX and X display the success of the respondents in the specialty certifying process. It seems reasonable to assume that successful candidates were more likely to return the questionnaire than were unsuccessful candidates. This would have a enhancing affect on the results. Those respondents who completed fellowship in 1981 or before could have been eligible for certification of special qualification in one of the three specialties at the time of the survey, and 64% of the respondents were in this category. The survey reveals that only 44% of the respondents have been certified

Table VIII. Certified in obstetrics and gynecology

	n	*Certified*	*Percent of respondents to this question*	*Percent of total respondents*
Maternal-fetal medicine	237	192	81	78.4
Reproductive endocrinology	186	151	81.2	78.6
Gynecologic oncology	152	129	84.9	83.2
Total	582*	477	82.0	79.6
Nonrespondents	17			

*Includes those not indicating subspecialty (n = 7).

for special qualification (Table IX). The percent certified in gynecologic oncology exceeds that for the other two specialties. There are a number of individuals who have passed the written examination and are no longer eligible to take the oral examination. Table X indicates that 86% of those who answered the question indicated that they had passed the written examination in the specialty. If we assume that those who did not respond to this question have not passed the written examination, then the figure drops to 80%. On the basis of the date of completing fellowship, it could be estimated that 92% were eligible to have passed the written.

The majority of individuals who entered a fellowship are now full-time faculty members (Table XI). Overall, 60.8% of 595 respondents indicate that their current employment is as a full-time faculty member (slightly less for gynecologic oncologists). Approximately 16% are employed full-time by a hospital other than the medical school and an equal number are in full-time private practice. For those completing fellowship during recent years, the percent in private practice is somewhat lower and the percents in full-time medical school and full-time hospital are both slightly greater. However, there is no great difference between those completing fellowship in 1976 and those completing in 1983.

Those who have taken fellowship training indicate that almost one half of their professional time is devoted to the clinical area of their specialty (Table XII). Gynecologic oncologists devote 60% of their time to oncology, whereas those in the other two specialties devote slightly less than half of their time to the specialty. Thus some portion of time is devoted to the breadth of the specialty by individuals with fellowship training.

The mean for the percent of time devoted to research is 15.9% while the mode and median time devoted to research is 10%. Those in reproductive endocrinology devote the greatest amount of time and those in gynecologic oncology the least amount of time to research. Most research appears to be clinical research. This is most apparent for those in gynecologic oncology. The percent of research time devoted to laboratory research is 13.3% for gynecologic oncology; 19.0% for maternal-fetal medicine and 29.1% for reproductive endocrinology, according to the response to this question. From this, it is possible to conclude that the percent of total professional time devoted to laboratory research is 1.4% for oncology; 3.0% for maternal-fetal medicine; and 6.0% for reproductive endocrinology. The response indicates that 56.6% spend no time in laboratory research.

Table IX. Certified in subspecialty

	n	*Certified*	%
Maternal-fetal medicine	245	96	39.2
Reproductive endocrinology	192	78	40.6
Gynecologic oncology	155	85	54.8
Total	599*	261	43.6

*Includes those not indicating subspecialty (n = 3).

One measure of research productivity is the number of research grants submitted. Experience with research grant applications is displayed in Tables XIII to XVII. The survey revealed that 32.8% of individuals have submitted research grants to the federal government and slightly fewer have submitted research grants to each of the other sources of funding (Table XIII). Thus a significant number of former fellows answered that they had not submitted a research grant application. Approximately 40% of the respondents indicated that they had filed no research grant applications since completion of training, and 85.8% have not had a research grant funded by the federal government. Specialists in reproductive endocrinology most frequently and specialists in gynecologic oncology least often have submitted research grant applications. Table XIV reveals that the 599 respondents have submitted 1478 research grant applications. The mean number of grants submitted per respondent is <1 to each of the five funding sources listed and 2.5 to all sources combined. However, the mean number of grants submitted, for the individuals submitting any grants, ranges from 2.29 for the federal government to 1.72 for the university (Table XV). Those who submit grants have submitted multiple grants over the years (mean 4.1).

The success of submitted research grants is quite re-

Table X. Passed written examination in subspecialty

	Yes	*Percent respondents to this question*	*Percent of total respondents*	*No*	*Nonrespondents*
Maternal-fetal medicine	184	81.1	75.1	43	18
Reproductive endocrinology	161	91.5	83.9	15	16
Gynecologic oncology	129	89.0	83.2	16	10
Total	479*	86.3	80.0	76	44
Nonrespondents	20				

*Includes those who did not indicate subspecialty (n = 7).

Table XI. Current employment of subspecialists

	*All**		*Maternal-fetal medicine*		*Reproductive endocrinology*		*Gynecologic oncology*	
	n	%	*n*	%	*n*	%	*n*	%
Full-time medical school	362	60.8	149	6.18	118	61.5	91	58.7
Full-time hospital†	97	16.3	49	20.3	21	10.9	27	17.4
Full-time private practice	96	16.1	33	13.7	36	18.8	25	16.1
Part-time teaching	20	3.4	6	2.5	10	5.2	4	2.6
Other	20	3.4	4	1.7	7	3.6	8	5.2
Nonrespondents	4		4		0		0	

*Includes those who did not indicate subspecialty (n = 7).
†Other than medical school.

Table XII. Time allocations: Percent of total professional time

	All	*Maternal-fetal medicine*	*Reproductive endocrinology*	*Gynecologic oncology*
Area of subspecialty	49.1	44.2	46.0	60.3
Normal obstetrics	9.6	16.8	6.3	2.5
Operative gynecology	9.5	5.8	10.4	14.0
Outpatient care	11.5	11.4	12.2	10.9
Research	15.9	15.9	20.5	10.5
Other	4.3	5.6	3.3	3.6

spectable, as seen in Tables XVI and XVII. Of all the grants submitted to the federal government, 63.5% were approved and 34.5% were funded. The success was greater for those grants submitted to other funding sources. Table XVII reveals that 809 grants have been funded at a dollar value per funded grant ranging from $37,812 for oncology to $67,295 for maternal-fetal medicine.

The former fellows were asked who should carry out most laboratory research in their specialty and responded as follows: Ph.D., 29.9%, M.D., 13%, M.D., Ph.D., 26.4%; whomever has the interest and skills, 30.6%. When asked if they were satisfied with the amount of time devoted to laboratory research, 48.5% responded yes. There was very little difference in the response from the three groups: maternal-fetal medicine, 50.2%; reproductive endocrinology, 47.3%; gynecologic oncology, 49.4%.

The mean number of papers published in refereed journals was 7.8 (Table XVIII). There were 23.9% who reported that they had published no papers. There was no trend identified with respect to the year in which the training was completed.

As was true for the chairmen, Table XIX indicates that the majority of respondents believe that the number of trainees in their specialty should remain stable or decrease. Very few recommended an increase in number of trainees.

Comment

Although response was good, it was not 100% and conclusions from the survey must be made with that in mind. Both questionnaires emphasized laboratory research. It is possible that those who did not respond were influenced in one way or another by that. The respondents did provide some very specific financial information relating to research.

In 1983 The Association of Professors of Gynecology and Obstetrics and The American College of Obstetricians and Gynecologists surveyed all chairmen of de-

Table XIII. Percent of individuals submitting research grant applications since completion of training

	All (%)	*Maternal-fetal medicine (%)*	*Reproductive endocrinology (%)*	*Gynecologic oncology (%)*
Federal government	32.8	35.7	38	22.6
Foundations	29.5	34	28.4	23.9
Industry	23.9	24	30	16.8
University	30.6	30	36.5	25.8
Practice revenue	6.7	7	8.3	4.5
All sources	59.7	62.8	63.7	51.6

Table XIV. Research grant applications submitted since completion of training (mean per individual)

	All		*Maternal-fetal medicine*		*Reproductive endocrinology*		*Gynecologic oncology*	
	Sum	*Mean*	*Sum*	*Mean*	*Sum*	*Mean*	*Sum*	*Mean*
Federal government	447	0.8	200	0.8	189	1.0	60	0.4
Approved		0.5		0.5		0.7		0.2
Funded		0.3		0.3		0.4		0.2
Foundations	325	0.6	167	0.7	97	0.5	61	0.4
Industry	322	0.6	136	0.5	137	0.6	49	0.3
University	311	0.5	105	0.4	137	0.7	69	0.4
Practice revenue	73	0.1	26	0.1	36	0.2	11	0.1
All sources	1478	2.5	634	2.6	594	3.1	250	1.6

partments of obstetrics and gynecology[1] and found the mean number of M.D. and Ph.D. faculty per department to be identical to results of this survey. The APGO/ACOG survey reported an increase in the number of subspecialty faculty and a decrease in general obstetrics and gynecology faculty during the preceding 3 years. This trend is also apparent from the Board study. From 1974 to 1984 the mean number of M.D. faculty per department increased by 6. In 1984 the mean number of faculty with Board-approved fellowship training was 5.1 and there were other subspecialty faculty without fellowship training. The APGO/ACOG survey identified 448 full-time faculty certified in one of the three specialty areas, whereas the current study identified 571 full-time faculty with approved fellowship training. The increase in number is greater for maternal-fetal medicine and reproductive endocrinology than for oncology. The 1983 survey identified 13 schools with no full-time faculty certified for special qualification and the Board survey identified only eight schools without trained subspecialists. Those certified early did not have Board-approved fellowship training and many former fellows are not yet certified.

Pearse et al.[1] reported a greater percent of certified subspecialists with full-time faculty appointments (reproductive endocrinology, 84%; maternal-fetal medicine, 78%; gynecologic oncology, 63%) than was found for former fellows in the board survey. The difference might be explained if those who achieve certification are employed more often as full-time faculty than those who are not certified. There is no other evidence for this.

Table XV. Research grant applications submitted (mean per individual for those submitting any grants)

	Mean
Federal government	2.3
Foundations	1.9
Industry	2.3
University	1.7
Practice revenue	1.8

The APGO/ACOG survey reported that research faculty needs were projected to increase by 53%, which certainly was not the case in the Board survey. In the APGO/ACOG survey, chairmen indicated a need to add approximately four full-time equivalent faculty positions per department for full-time research by 1988. The Board survey indicated plans to recruit approximately two new faculty during the next 10 years who will devote more than 50% of their time to laboratory research (one full-time equivalent). This may reflect the difference between plans for laboratory research and clinical research and also the impact of the growing need to finance departments from clinical practice revenue.

Although former fellows devote some time to the breadth of the discipline, the majority of their time is devoted to their specialty area. Whereas essentially all

Table XVI. Percent of total submitted grants approved and funded

	All	*Maternal-fetal medicine*	*Reproductive endocrinology*	*Gynecologic oncology*
Federal government				
Approved	63.5	61	70	51.7
Funded	34.5	30	36.9	43.3
Foundation				
Approved	62.5	58	66	68.9
Funded	49.9	46	53.6	54.1
Industry				
Approved	87.0	92	84.7	79.6
Funded	82.6	86.7	81.8	73.5
University				
Approved	73.6	71.4	81.8	60.9
Funded	56.6	51	67.8	42
Practice revenue				
Approved	79.5	73	77.8	100
Funded	69.9	54	77.8	81.8

Table XVII. Research funding from all sources since completion of fellowship

	Maternal-fetal medicine	*Reproductive endocrinology*	*Gynecologic oncology*
No. of grants submitted	634	594	250
No. of grants approved	438	451	165
No. of grants funded	322	354	133
Amount per funded grant	$67,295	$46,839	$37,812
Amount per submitted grant	$34,178	$27,914	$20,116
Total amount	$21,669,000	$16,581,000	$5,029,000

subspecialists who are eligible have been certified in obstetrics and gynecology and most have passed the specialty written examination, it may be surprising that only 44% of the respondents are certified in their subspecialty. The requirement of a current published article influences that. Recent Board policy changes may decrease the number of individuals who pass the specialty written examination but never achieve eligibility for the oral examination.

The future need for diplomates certified for special qualification is uncertain. The response from both chairmen and subspecialists does not indicate a strong desire to increase the number of fellows in training, although some chairmen indicate a need for increased numbers. The 1983 APGO/ACOG survey projected a need for 414 new subspecialists by 1988: 164 in maternal-fetal medicine, 114 in gynecologic oncology, and 136 in reproductive endocrinology. Such numbers could be met by those currently being certified only if nearly all accepted full-time faculty appointments. In 1972 the American Board of Obstetrics and Gynecology surveyed all certified obstetrician-gynecologists in the United States. In that survey,[2] 14.1% of 9297 respondents indicated that there was an area of obstetric-gynecologic practice in which a majority of their practice time and effort was being devoted, and 10.2% indicated one of the three current divisions (maternal-fetal medicine, 4.7%; gynecologic oncology, 2.9%; and reproductive endocrinology, 2.6%). By 1985 the 896 diplomates with certificates of special qualification represents 3.9% of the approximately 22,700 living diplomates. Certificates of special qualification were issued to 158 diplomates in 1984 and 1985, which represents 7.2% of diplomates certified in the same years. The number of fellows graduating in 1985 and 1986 represents 12.8% of the total number of residents who graduated in 1983 and 1984. This suggests that current subspecialty training approximates practice need. There have been no formal studies of the demand for subspecialists, but estimates based on the 1972 board survey,[2] identified patient problems in the state of Colorado (E. S. Taylor), and projections for development of tertiary care centers (W. H. Pearse) each have suggested a need for 300 to 400 specialists in reproductive endocrinology and gynecologic oncology and 700 to 800 in maternal-fetal medicine. There has never been an effort to establish a desired number of specialty fellows. Despite that, the number of fellows graduating annually has plateaued and is approximately what several have estimated the need to be. It is possible that such estimated needs will be met by the end of the century.

One objective of the questionnaire was to determine the impact of subspecialization on research, with em-

Table XVIII. Articles published in refereed journals since completion of fellowship

No. of articles	*Maternal-fetal medicine*		*Reproductive endocrinology*		*Gynecologic oncology*	
	n	%	*n*	%	*n*	%
0	59	24	53	28	27	17
1-5	96	40	63	33	57	37
6-10	28	12	35	18	27	17
11-20	35	15	19	10	24	15
21-30	12	5	8	4	11	7
41-50	4	2	3	2	2	1
51-60	1	–	3	2	1	1
61-70	–	–	–	–	–	–
71-80	–	–	–	–	1	1
Mean No. of articles per respondent	7.4	–	7.7	–	8.8	–

Table XIX. Recommended change in fellowship trainees (percent of subspecialist respondents)

	Increase	*Decrease*	*Remain stable*
Maternal-fetal medicine	15.5	14.2	70.3
Gynecologic oncology	2.6	45.1	52.3
Reproductive endocrinology	13.5	20.5	65.9
All	11.8	24.3	63.9

phasis on laboratory research. The median and mode for percent of professional time devoted to research was 10% with a mean of 15.9%. The mean for reproductive endocrinology was higher (20.5%). Most research is clinical research. The percent of total professional time devoted to laboratory research varied from 1.4% for oncology to 6% for reproductive endocrinology. Although 40% of respondents have never filed a research grant application, the success of the many submitted research grants is good. Of all research grants submitted to the federal government, for example, 34.5% were approved and funded. Total research funding to these former fellows is $43,279,000.00. Certainly this has increased the research productivity of medical school departments of obstetrics and gynecology.

Thus the survey reveals that research is not the major activity of subspecialists. Undoubtedly there are many reasons for this and one may be the nature of those who have entered obstetrics and gynecology. The Board studied the scholastic ability of those who finished obstetrics and gynecology residencies in 1983 and 1984. To the extent that scholastic ability is measured by performance on National Board of Medical Examiners Part I and Part II examinations, residents in obstetrics and gynecology, on the average, had slightly less of that ability than national norm groups who took the same examinations.

Ten years after the first certificates of special qualification were granted, it appears that the objectives of this new certification by the Board are being achieved. Qualified physicians have been recruited into the special areas of obstetrics and gynecology and more than 60% have full-time medical school faculty appointments. Almost all departments have one or more trained subspecialists. The fellowship training programs are regularly reviewed and have structured required curricula to ensure appropriate training of fellows. Those who have been trained devote more than half of their time to the specialty area but most are not removed from the principal discipline. The development of divisions within departments of obstetrics and gynecology has elevated the standards of education in each of the special areas and contributes to improvement in the organization and distribution of patient services. Although subspecialists devote most of their time to clinical activities, they are involved in laboratory research that increases basic knowledge in the special areas. It is difficult to separate the impact of newly emerging technology and knowledge from the impact of advanced training.

I gratefully acknowledge the assistance of Doug Schaad, M.Ed., Division of Research in Medical Education, University of Washington School of Medicine, Seattle, Washington.

REFERENCES

1. Pearse WH, Davidson EC, Fielden JG. Trends in obstetric-gynecologic academic manpower—1983. Obstet Gynecol 1985;65:147.
2. Randall CL. The current practices of board-certified obstetricians and gynecologists in the United States. Am J Obstet Gynecol 1974;119:156.

The diagnostic value of hysterosalpingography and hysteroscopy in infertility investigation

Jamil A. Fayez, M.D., Ghazi Mutie, M.D., and Peter J. Schneider, M.D.
Winston-Salem, North Carolina

Four hundred infertile patients had hysterosalpingography and hysteroscopy as part of their infertility workup. A comparison between the findings of these two procedures was carried out to study their diagnostic value in female infertility investigation. It was found that hysterosalpingography was as accurate as hysteroscopy in the diagnosis of normal or abnormal uterine cavities while the nature of the intrauterine filling defects was accurately revealed by hysteroscopy only. It is concluded that hysterosalpingography is an important screening procedure for the diagnosis of normal or abnormal uterine cavities and that hysteroscopy should be reserved only for the confirmation and treatment of intrauterine anomalies discovered by hysterosalpingography. Therefore we look at the two procedures, hysterosalpingography and hysteroscopy, as complementary techniques. (AM J OBSTET GYNECOL 1987;156:558-60.)

Key words: Hysterosalpingography, hysteroscopy, infertility

Hysteroscopy was first performed more than 100 years ago,[1] but the procedure was practiced by few physicians until recently. With advances in instrumentation and the availability of better distention media, interest in hysteroscopy has increased, particularly with regard to its applicability in the investigation of female infertility.[2] Reports on the diagnostic value of hysteroscopy are conflicting, with many authors claiming increased accuracy of hysteroscopy compared with hysterosalpingography in the diagnosis of intrauterine lesions.[3, 4] Some authors[5, 6] have suggested that hysterosalpingography should be completely supplanted by endoscopy. In this context, it was thought to be appropriate to make this retrospective study about hysterosalpingography and hysteroscopy to determine the diagnostic value of each procedure and to establish certain criteria whereby each might be properly used.

Material and methods

The study population consisted of 400 women between the ages of 17 and 40 (mean 29.2) who had a complete infertility workup, including hysterosalpingography and hysteroscopy. Hysteroscopy was performed routinely for most infertile patients who had diagnostic or operative laparoscopy. Whether diagnostic or operative, hysteroscopy always followed laparoscopy to avoid instillation of contrast medium into the pelvis, which could have interfered with any operative laparoscopy procedures. All patients who had operative hysteroscopy were included in this study while those who had diagnostic procedures were selected randomly. All charts were reviewed to compare the diagnostic value of hysterosalpingography and hysteroscopy in the investigation of female infertility. Hysterosalpingography preceded hysteroscopy by 1 to 6 months in all cases; the interval depended on the indication of hysteroscopy on the basis of history and physical examination as well as the findings of hysterosalpingography.

All hysterosalpingographic examinations were outpatient procedures and they were performed and interpreted by a reproductive endocrinologist in conjunction with a radiologist. They were performed in the proliferative phase of the menstrual cycle with sterile techniques and without analgesia or anesthesia. Water-soluble contrast medium, 10 to 20 ml (Sinografin, E. R. Squibb & Sons, Princeton, New Jersey), was injected slowly into the cervical canal via a plastic acorn with the aid of fluoroscopy monitoring. In the majority

Table I. Indications for hysteroscopy

Indication	*No.*	*%*
Routine infertility workup	177	44.25
Unexplained infertility workup	43	10.75
Intrauterine synechiae	96	24.0
Intrauterine filling defects other than synechiae	26	6.5
Double uterus (septate or bicornuate)	24	6.0
Diethylstilbestrol-associated uterus	34	8.5
Total	400	100.0

From the Section of Reproductive Endocrinology, Bowman Gray School of Medicine of Wake Forest University.
Received for publication March 10, 1986; revised September 8, 1986; accepted September 30, 1986.
Reprint requests: Jamil A. Fayez, M.D., Section on Reproductive Endocrinology, Bowman Gray School of Medicine, 300 South Hawthorne Road, Winston-Salem, NC 27103.

Table II. Uterine findings at hysterosalpingography and hysteroscopy

Uterine finding	*Hysterosalpingography*	*Hysteroscopy*
Normal uterine cavity	194	219 (194 + 25)
Synechiae	96	82
Arcuate uterus	26	20
Double uterus (septate or bicornuate)	24	24
Polyps	16	9
Fibroids	10	12 (10+ 2)
Diethylstilbestrol-associated uterus	34	34
Total	400	400

of cases two anteroposterior films were sufficient to allow the diagnosis of uterine or tubal abnormalities.

Hysteroscopy was performed in the proliferative phase of the menstrual cycle with the patient under general anesthesia with endotracheal intubation. Although local anesthesia is adequate for diagnostic hysteroscopy, it was not feasible here as all hysteroscopy procedures were combined with diagnostic or operative laparoscopy. Hyskon (Pharmacia Laboratories, Inc., Piscataway, New Jersey) was the only distention medium used with an average of 40 ml needed for diagnostic purposes and 150 ml for operative procedures. Once the uterine cavity was adequately distended, a systemic exploration was performed. The fundus was examined first; then the anteroposterior and lateral walls were scrutinized. Before the procedure was completed, both ostia were identified and the cervical canal was thoroughly scanned. Findings were recorded on a hysteroscopic examination form for future review. The indications for hysteroscopy in this study are shown in Table I.

Results

Synechiae were easily identified by hysteroscopy as bridges extending from one part of the uterine cavity to another. The arcuate uterus was identified hysteroscopically by the shallow and smooth fundal wedge protruding into the uterine cavity. The septate and bicornuate uteri were differentiated by the laparoscopic appearance of their fundi and then examined hysteroscopically. The septum in the septate uterus appeared triangular with a thin apex in contradistinction with the thick, broad and short muscular partition in the bicornuate uterus. All of the 12 fibroids were single and sessile. Nine of these were ≤ 3 cm and were extracted hysteroscopically with bleeding minimized by the pressure of the bulb of a Foley catheter. The other three fibroids were ≥ 5 cm and were removed abdominally. When the 34 diethylstilbestrol-associated uteri diagnosed by hysterosalpingography were examined hysteroscopically, they showed different abnormalities including small uterine cavities, multiple short synechiae, intracervical ridges, and short or long cervical canals.

Table II shows that hysterosalpingography was as accurate as hysteroscopy in the diagnosis of normal uterine cavities, double uteri (septate and bicornuate), and diethylstilbestrol-associated uteri but was inaccurate in 27 cases belonging to the remaining subgroups. Hysterosalpingography showed synechiae in 96 patients while hysteroscopy confirmed the diagnosis in only 82. Sixteen patients were diagnosed as having polyps; five of these proved to be mucus or thick shaggy endometrium when visualized by hysteroscopy and two were small fibroids. A χ^2 test showed that there was a significant difference in the accurate diagnosis of uterine synechiae when made by hysteroscopy ($p \leq 0.002$) while the difference was not significant in the diagnosis of other uterine anomalies made by the two procedures ($p \geq 0.05$).

Comment

Procedures to determine whether a uterine factor is responsible for infertility include endometrial sampling, endometrial culture, hysterosalpingography, hysteroscopy, and laparoscopy. Hysterosalpingography is a simple, noninvasive procedure that enables visualization of the uterine cavity contour, revealing or ruling out anomalies that include different types of intrauterine defects. For centuries, hysterosalpingography was the sole procedure that could provide adequate information about the normality or abnormality of the uterine cavity. Hysteroscopy, the direct visualization of the interior of the uterine cavity, was, until the early 1970s, of controversial value. Most recently, hysteroscopy has developed into a highly sophisticated and effective technique with increasing applicability. While studies[5,6] have demonstrated the increased accuracy of hysteroscopy over hysterosalpingography in the diagnosis of intrauterine lesions, others found that hysterosalpingography was more reliable.[4] Over the years, we have been impressed with the positive correlation between the findings of hysterosalpingography and those of hysteroscopy. Therefore this retrospective study was made to compare the diagnostic value of each of these two techniques in view of the conflicting reports regarding which procedure has the advantage over the other.

The results of this study demonstrate that both hysterosalpingography and hysteroscopy constitute valuable methods in the investigation of infertility. Table II shows that there is no statistical difference in the diagnosis of uterine abnormalities by either hysterosalpingography or hysteroscopy. The conflict between the results of this study and those of others is probably due to difference in techniques and interpretations. Our patients were thoroughly counseled regarding the details of hysterosalpingography to reduce tension that would have contributed to uterine spasm. It is known that contrast material may induce localized uterine spasm that may resemble an intrauterine defect.[7] This problem was avoided in this study by injecting the dye slowly but steadily without interruption until the procedure was completed. In contrast to many practices where hysterosalpingography is performed and interpreted by radiologists, ours were performed or supervised and interpreted by the senior author (J. A. F.) in conjunction with a radiologist. All these precautionary measures might have contributed to the correct diagnosis obtained by hysterosalpingography in this study.

Differential diagnosis of an intrauterine filling defect includes air bubbles, mucus, polyps, submucous fibroids, intrauterine synechiae, a septum, and, rarely, asynchronous uterine contractions. Hysterosalpingography can easily detect these abnormalities and in the majority of cases reveals the nature of the intrauterine defects as shown in Table II. The few discrepancies between the diagnosis obtained by hysterosalpingography and those obtained by hysteroscopy are expected since hysterosalpingography localizes the defects while hysteroscopy makes them directly visualized. An average hysteroscopist may confuse one type of intrauterine defect with another. Endometrial fragmentation or incomplete shedding can mimic synechiae. Extra care must be taken to differentiate polyps from thick shaggy endometrium or from myomas. The polyp has a rounded, smooth free end, while thick strips of endometrium have a shredded appearance and can be easily dislodged by gentle strokes with the end of the hysteroscope. Myomas can be easily distinguished from polyps as the latter have gentle undulations as the pressure of the distending medium varies.

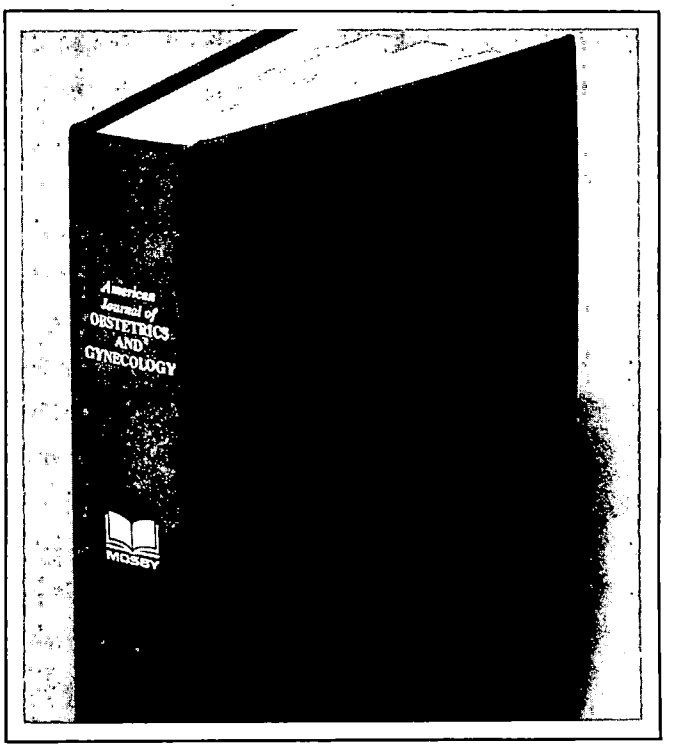
It is obvious from this study that hysterosalpingography is very reliable in the diagnosis of uterine abnormalities. In addition to this diagnostic value, hysterosalpingography does provide information about tubal patency or blockage. Another benefit of hysterosalpingography that most gynecologists must have experienced is the therapeutic effect of hysterosalpingography.[8]

Because hysterosalpingography is a much less invasive procedure than hysteroscopy and because of the valuable information it provides about the uterine cavity and tubes, hysterosalpingography should be mandatory in the evaluation of infertility and recurrent pregnancy loss. When an intrauterine abnormality is detected, it is obvious that the nature of the lesion should be identified by direct visualization and dealt with by operative hysteroscopy. If hysterosalpingography fails to demonstrate any intrauterine abnormality, then hysteroscopy becomes a controversial issue. We believe that hysteroscopy should not be considered as a part of the routine infertility workup, but, after a complete infertility workup and when no apparent cause is found, laparoscopy combined with hysteroscopy is essential for the completion of the investigation. The yield of the combined approach in patients with unexplained infertility is high.[9]

REFERENCES

1. Pantaleoni D. An endoscopic examination of the cavity of the womb. Med Press Circ 1869;8:26.
2. Valle RF, Sciarra JJ. Hysteroscopy: a useful diagnostic adjunct in gynecology. Am J Obstet Gynecol 1975;122:230.
3. Valle RF. Hysteroscopy in the evaluation of female infertility. Am J Obstet Gynecol 1980;137:425.
4. Snowden EU, Jarrett JC, Dawood M-Y. Comparison of diagnostic accuracy of laparoscopy, hysteroscopy, and hysterosalpingography in evaluation of female infertility. Fertil Steril 1984;41:709.
5. Maathius JB, Harbach JG, Van Hall EV. A comparison of the results of hysterosalpingography and laparoscopy in the diagnosis of fallopian tube dysfunction. Fertil Steril 1972;23:428.
6. Taylor PJ. Correlations in infertility: symptomatology, hysterosalpingography, laparoscopy and hysteroscopy. J Reprod Med 1977;8:339.
7. Siegler AM. Hysterosalpingography. Fertil Steril 1983;40:139.
8. Gillespi HW. The therapeutic aspect of hysterosalpingography. Br J Radiol 1986;38:301.
9. Taylor PJ, Leader A, George RE. Combined laparoscopy and hysteroscopy in the investigation of infertility in hysteroscopy. In: Siegler AM, Lindemann HJ, eds. Principles and practice. Philadelphia: JB Lippincott, 1983:207.

Here's what MOSBY'S BOUND VOLUMES can add to your practice!



MOSBY
TIMES MIRROR

Handsomely bound in durable buckram with rich gold stamping, bound journal reference volumes are important to every successful practice.

- gives quick and easy access to important journal articles
- eliminates problems with missing issues or pages
- makes your regular subscription a "working" copy to mark up, tear from, or file
- offers an inexpensive and easy way to guarantee bound reference volumes—prices include shipping

You Must Order Now!
Bound volumes are made up from over-run copies of each journal issue. You never worry about sending out your personal copies. But over-run quantities are limited. We need your order today! Bound volumes are only available to current subscribers and may not be ordered in place of a regular journal subscription. Payment must accompany your order.

Credit Card Orders In The U.S. Call FREE:
1-800-325-4177, ext. 351
(In Missouri, call collect: (314) 872-8370, ext. 351)

YES! Send me the 1987 bound volumes of:

☐ *AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY*

Jan.-June, 1987 (Vol. 156, No. 1-6)
July-Dec., 1987 (Vol. 157, No. 1-6)

Total 1987 Price, Two Volume Set

USA	International
$51.00	$74.00

All orders must include payment. Bound volumes are non-returnable.

My check for $_____ is enclosed.

☐ VISA ☐ MasterCard

Card No._____

Exp. date_____

Name_____
(Please Print)

Address_____

_____

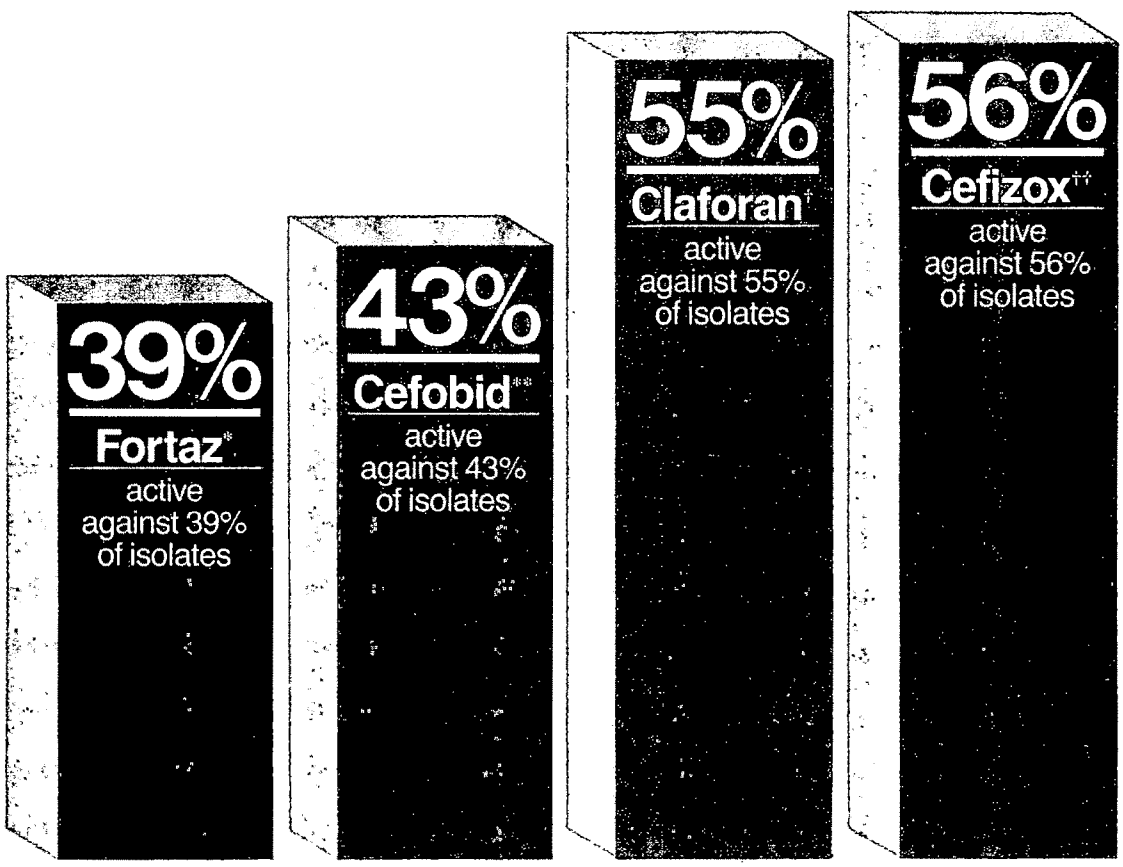
City_____

State_____ Zip_____

Clip and return coupon to: The C.V. Mosby Company, Attn: Bound Volumes, 11830 Westline Industrial Dr., St. Louis, MO 63146. Outside the U.S. please pay by check drawn on a U.S. bank in U.S. dollars or use credit card. **ABS-127**

Why settle for less activit

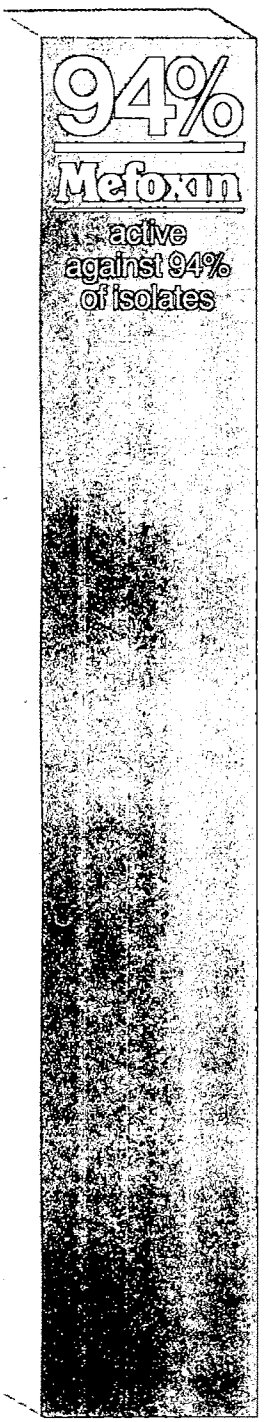
Don't Compromise



*Fortaz is a registered trademark of Glaxo Inc.
**Cefobid is a registered trademark of Pfizer Inc.
†Claforan is a registered trademark of Roussel-Uclaf.
††Cefizox is a registered trademark of Fujisawa SmithKline Corporation.

Copyright © 1987 by Merck & Co., Inc.

gainst the *B. fragilis* group?



the facts still favor Mefoxin® IV/IM (Cefoxitin Sodium | MSD)

The *Bacteroides fragilis* group includes *B. fragilis, B. distasonis, B. ovatus, B. thetaiotaomicron, B. vulgatus.*

The percentages displayed are a weighted average of the susceptibilities reported for these organisms at an MIC breakpoint of 16 mcg/mL by the Antibiotic MIC Susceptibility Reports, National Summary, in *The Bacteriologic Report: Volume I 1986,* Totowa, NJ, BAC-DATA® Medical Information Systems, Inc. This average was weighted by the frequency of isolation of each organism.

NOTE: *In vitro* activity does not necessarily imply *in vivo* effectiveness.

MEFOXIN is contraindicated in patients who have shown hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

MEFOXIN is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Streptococcus faecalis*) and many strains of *Enterobacter cloacae.* Methicillin-resistant staphylococci are almost uniformly resistant to MEFOXIN.

For a Brief Summary of Prescribing Information, please see following page.

MSD
MERCK SHARP & DOHME

Mefoxin® IV/IM (Cefoxitin Sodium | MSD)

Indications and Usage: *Treatment*—Serious infections caused by susceptible strains of the designated microorganisms in the following diseases:

LOWER RESPIRATORY TRACT INFECTIONS, including pneumonia and lung abscess, caused by *Streptococcus pneumoniae* (formerly *Diplococcus pneumoniae*), other streptococci (excluding enterococci, e.g., *Strep. faecalis*), *Staphylococcus aureus* (penicillinase and non-penicillinase producing), *Escherichia coli, Klebsiella* species, *Hemophilus influenzae*, and *Bacteroides* species.

GENITOURINARY INFECTIONS. Urinary tract infections caused by *E. coli, Klebsiella* species, *Proteus mirabilis*, indole-positive *Proteus* (i.e., *P. morganii, P. rettgeri*, and *P. vulgaris*), and *Providencia* species. Uncomplicated gonorrhea due to *Neisseria gonorrhoeae* (penicillinase and non-penicillinase producing).

INTRA-ABDOMINAL INFECTIONS, including peritonitis and intra-abdominal abscess, caused by *E. coli, Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ and *Clostridium* species.

GYNECOLOGICAL INFECTIONS, including endometritis, pelvic cellulitis, and pelvic inflammatory disease, caused by *E. coli, N. gonorrhoeae* (penicillinase and non-penicillinase producing), *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and group B streptococci.

SEPTICEMIA caused by *Strep. pneumoniae* (formerly *D. pneumoniae*), *Staph. aureus* (penicillinase and non-penicillinase producing), *E. coli, Klebsiella* species, and *Bacteroides* species including the *B. fragilis* group.§

BONE AND JOINT INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing).

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis*, streptococci (excluding enterococci, e.g., *Strep. faecalis*), *E. coli, P. mirabilis, Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae*. Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.

Contraindications: Previous hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

Warnings: BEFORE THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOXITIN, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. GIVE WITH CAUTION TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. IF AN ALLERGIC REACTION TO CEFOXITIN OCCURS, DISCONTINUE THE DRUG. SERIOUS HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

Pseudomembranous colitis, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use. Broad-spectrum antibiotics alter normal flora of colon and may permit overgrowth of clostridia; a toxin produced by *Clostridium difficile* is a primary cause of antibiotic-associated colitis. Mild cases may respond to drug discontinuance alone; in more severe cases, management may include sigmoidoscopy, appropriate bacteriological studies, fluid, electrolyte and protein supplementation, and use of a drug such as oral vancomycin; isolation of the patient may be advisable. Other causes of colitis should also be considered.

Precautions: *General*—Total daily dose should be reduced in patients with reduced urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur from usual doses. Prescribe with caution in patients with a history of gastrointestinal disease, particularly colitis. Prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures.

Drug Interactions—Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.

Drug/Laboratory Test Interactions—High concentrations (>100 mcg/mL) may interfere with measurement of serum and urine creatinine levels by the Jaffé reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. High concentrations may interfere with measurement of urinary 17-hydroxy-corticosteroids by the Porter-Silber reaction and produce false increases of modest degree in levels reported. A false-positive reaction for glucose in urine has been observed with CLINITEST II reagent tablets.

Carcinogenesis, Mutagenesis, Fertility Impairment—No long-term animal study has been performed on carcinogenic or mutagenic potential. Rat studies at approximately three times maximum recommended human dosage revealed no effects on fertility or mating ability.

Pregnancy Category B—Reproduction studies in rats and mice did not reveal teratogenic or fetal toxic effects, although fetal weights were slightly decreased. In rabbits, cefoxitin was associated with a high incidence of abortion and maternal death, neither considered teratogenic. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers—Excreted in human milk: Exercise caution.

Pediatric Use—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.

Adverse Reactions: The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently. *Local Reactions*—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. *Allergic Reactions*—Rash (including exfoliative dermatitis), pruritus, eosinophilia, fever, and other allergic reactions including anaphylaxis. *Cardiovascular*—Hypotension. *Gastrointestinal*—Diarrhea, including documented pseudomembranous colitis during or after treatment, and, rarely, nausea and vomiting. *Blood*—Eosinophilia, leukopenia including granulocytopenia, neutropenia, anemia, including hemolytic anemia, thrombocytopenia, and bone marrow depression. A positive direct Coombs test may develop in some individuals, especially those with azotemia. *Liver Function*—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase. *Renal Function*—Elevations in serum creatinine and/or blood urea nitrogen levels and, rarely, acute renal failure.

Note: In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

How Supplied: Sterile cefoxitin sodium in vials and infusion bottles containing 1 gram or 2 grams cefoxitin equivalent and in 10-gram bulk bottles, and in ADD-Vantage®† vials containing 1 gram or 2 grams cefoxitin equivalent.

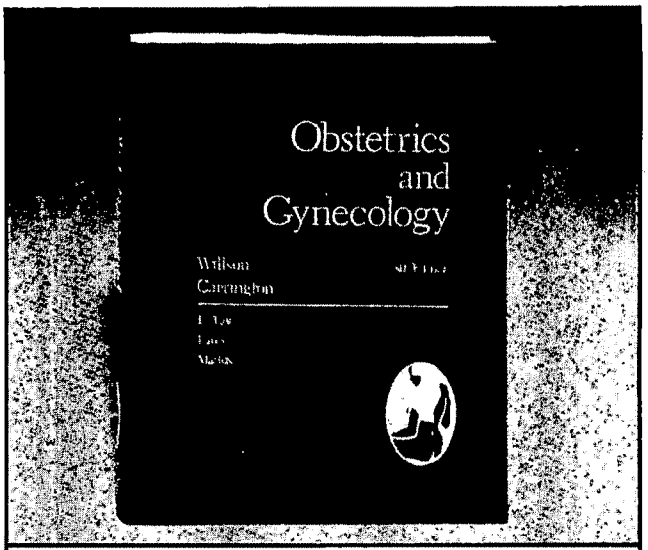
§ *B. fragilis, B. distasonis, B. ovatus, B. thetaiotaomicron, B. vulgatus*

‖ Registered trademark of Ames Company, Division of Miles Laboratories, Inc.

†Registered trademark of Abbott Laboratories, Inc.

For more detailed information, consult your MSD Representative or see Prescribing Information. Merck Sharp & Dohme,

MSD
MERCK SHARP & DOHME



New 8th Edition! OBSTETRICS AND GYNECOLOGY

Edited by J. Robert Willson, M.D. and Elsie Reid Carrington, M.D.; Associate Editors Russell K. Laros, Jr., M.D.; William J. Ledger, M.D.; and John H. Mattox, M.D.; with 4 contributors.

In a review of the 7th edition, JAMA said that it "maintains excellence especially in general obstetrics" and "fulfills its purpose as a useful text for various levels of students of obstetrics and gynecology." This new edition, with the addition of two new editors, continues the tradition! Emphasis is on treatment of the "total woman," following the normal life-cycle—birth through pregnancy.

- easy-to-read format facilitates Board review
- a new chapter on androgen disorders
- infertility, endocrinology and endometriosis
- ectopic pregnancy, abortion and infectious disease
- radiologic studies in pregnant women

April, 1987. Approx. 768 pages, 562 illustrations. (Book Code: 05650) About $44.95 (U.S.); $62.25 (Can.)

In a hurry to order? Call FREE 800-221-7700, ext. 15A. Our hotline is open 24 hours a day, 7 days a week. In Canada call 416-298-1588.

MOSBY TIMES MIRROR

AMS-970-031

Yes! Send my 30-day on-approval copy of: Willson's OBSTETRICS AND GYNECOLOGY (05650) about $44.95 (U.S.); $62.25 (Can.)

Save money! Enclose payment with your order or charge it to your credit card. Mosby will pick up the tab for shipping and handling.

☐ Payment enclosed

Charge my credit card: ☐ MasterCard ☐ VISA

Card # _____ Exp. date _____

Signature _____

Business phone (_____) _____

☐ Bill me, plus shipping and handling.

Name _____

Address _____

City _____

State _____ Zip _____

☐ Add me to your mailing list. (No purchase necessary)

Specialty _____

To properly process Purchase Orders and for more personal service, mail this coupon to: Dennis Carson, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, MO 63146. 30-day approval good in U.S. and Canada. Outside U.S. and Canada, please pay in dollar equivalent. ALL PRICES SUBJECT TO CHANGE. Add applicable sales tax.

AMS-970-031-03



Now

The next generation of estrogen replacement therapy...

transdermal delivery

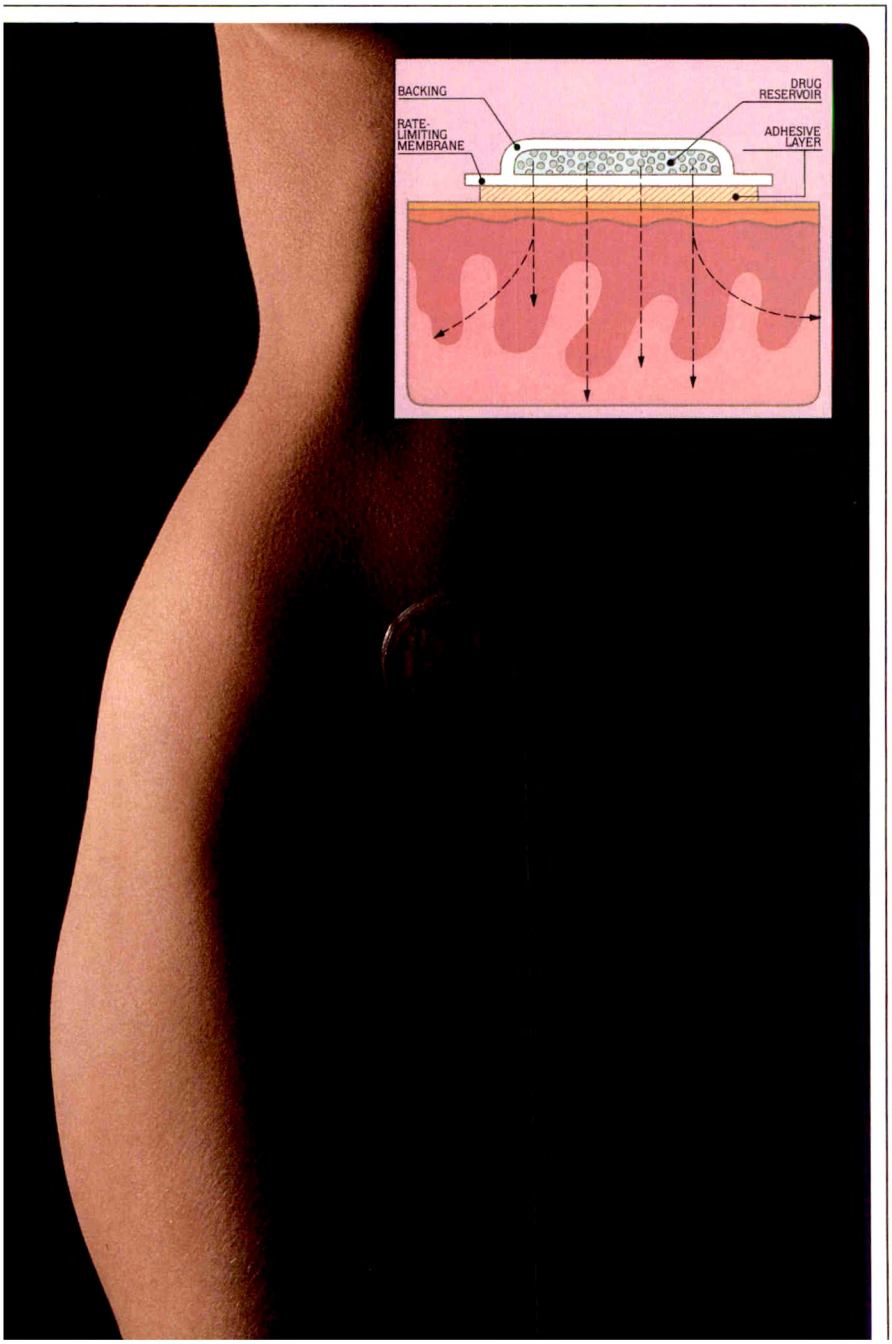


NEW

ESTRADERM®

estradiol transdermal syster

Combines the advantages of transdermal delivery...



A specially designed transdermal delivery system

Providing the natural ovarian estroge (17-beta estradiol) directly to the bloodstream and target organs; with no first-pass liver metabolism, permi use of extremely low dosage

Effectively controls estrogen deficiency symptoms

Steady-state relief of hot flashes, oth menopausal symptoms associated wi hot flashes, and vaginal discomfort

Well tolerated

Very low incidence of systemic side effects...few local problems of consequence

Easy to use; transdermal delivery preferred 3:1 by patients over tablets[1]

Usual starting dose: a 0.05-mg syste changed twice weekly

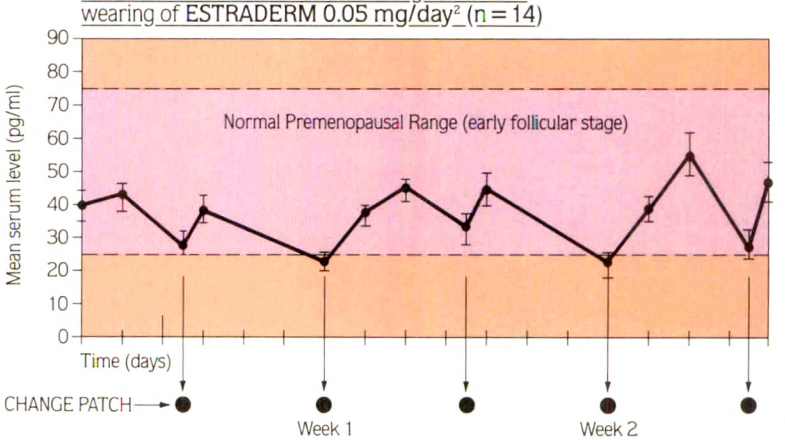
C I B A

ith the benefits of strogen replacement

stores and maintains premenopausal tradiol serum levels

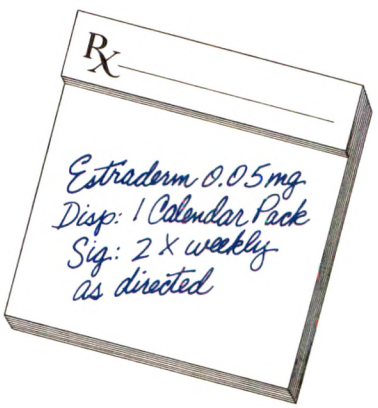
tores estradiol levels to near-normal premenopausal ranges
chieves a physiologic 1:1 ratio of estradiol to estrone

Mean serum levels of estradiol during continuous wearing of ESTRADERM 0.05 mg/day[2] (n = 14)





lable in dose-reminder calendar packs of eight
hes—0.05 mg/0.1 mg



References:

1. Data on file, CIBA Pharmaceutical Company.
2. Powers MS, et al: Pharmacokinetics and pharmacodynamics of transdermal dosage forms of 17 β-estradiol: Comparison with conventional oral estrogens used for hormone replacement. Am J Obstet Gynecol 1985; 152:1099–1106.

I B A

ing today's women live healthier lives

Please see next page
for Brief Summary of Prescribing Information.

ESTRADERM®

estradiol transdermal system

Continuous delivery for twice-weekly application.
BRIEF SUMMARY OF PRESCRIBING INFORMATION.
PLEASE SEE FULL PRESCRIBING INFORMATION.

ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than 1 year. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer-reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5-13.9 times greater than in nonusers. The risk appears to depend both on duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.

The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that women who had been exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than four per 1000 exposures. Furthermore, a high percentage of such exposed women (30-90%) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data on the use of other estrogens are not available, it cannot be presumed they would not induce similar changes.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb-reduction defects. One case control study estimated a 4.7-fold increased risk of limb-reduction defects in infants who had been exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses.

If Estraderm is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus and of the advisability of continuation of the pregnancy.

INDICATIONS AND USAGE

Estraderm is indicated for the treatment of the following: moderate-to-severe vasomotor symptoms associated with menopause; female hypogonadism; female castration; primary ovarian failure; and atrophic conditions caused by deficient endogenous estrogen production, such as atrophic vaginitis and kraurosis vulvae.

Studies of the addition of a progestin for seven or more days of a cycle of estrogen administration have reported a lowered incidence of endometrial hyperplasia. Morphological and biochemical studies of endometrium suggest that 12 to 13 days of progestin are needed to provide maximal maturation of the endometrium and to eliminate any hyperplastic changes. Whether this will provide protection from endometrial carcinoma has not been clearly established. There are possible additional risks that may be associated with the inclusion of progestin in estrogen replacement regimens. The potential risks include adverse effects on carbohydrate and lipid metabolism. The choice of progestin and dosage may be important in minimizing these adverse effects.

CONTRAINDICATIONS

Estrogens should not be used in women or men with any of the following conditions:

1. known or suspected cancer of the breast;
2. known or suspected estrogen-dependent neoplasia;
3. known or suspected pregnancy (see Boxed Warning);
4. undiagnosed abnormal genital bleeding;
5. active thrombophlebitis or thromboembolic disorders;
6. history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use.

WARNINGS

1. Induction of Malignant Neoplasms. Long-term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. There are now reports that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning.)

At the present time, there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of breast cancer, although a recent long-term follow-up of a single physician's practice has raised this possibility. Because of the animal data, there is a need for caution in prescribing estrogens for women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms.

2. Gallbladder Disease. A recent study has reported a two- to threefold increase in the risk of surgically confirmed gallbladder disease in postmenopausal women receiving oral estrogens, similar to the twofold increase previously noted in users of oral contraceptives.

3. Effects Similar to Those Caused by Estrogen-Progestogen Oral Contraceptives. There are several serious adverse effects of oral contraceptives and other high-dose oral estrogen treatments, most of which have not, up to now, been documented as consequences of postmenopausal estrogen replacement therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women.

a. Thromboembolic Disease. It is now well established that users of oral contraceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least four weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization.

While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found, this does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore, estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be used in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

b. Hepatic Adenoma. Benign hepatic adenomas have been associated with the use of oral contraceptives. Although benign and rare, these tumors may rupture and cause death from intra-abdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations, but they should be considered if abdominal pain and tenderness, abdominal mass, or hypovolemic shock occurs in patients receiving estrogen. Hepatocellular carcinoma has also been reported in women taking estrogen-containing oral contraceptives. The causal relationship of this malignancy to these drugs is not known.

c. Elevated Blood Pressure. Women using oral contraceptives sometimes experience increased blood pressure which, in most cases, returns to normal upon discontinuing the drug. There is now a report that this may occur with use of oral estrogens in the menopause and blood pressure should be monitored with estrogen use, especially if high doses are used. Ethinyl estradiol and conjugated estrogens have been shown to increase renin substrate. In contrast to these oral estrogens, transdermally administered estradiol does not affect renin substrate.

d. Glucose Tolerance. A worsening of glucose tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen.

4. Hypercalcemia. Administration of high doses of estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases. If hypercalcemia occurs, use of the drug should be stopped and appropriate measures should be taken to reduce the serum calcium level.

PRECAUTIONS

General

1. A complete medical and family history should be taken before initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, as well as a cervical Papanicolaou test. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed.
2. Because estrogens may cause some degree of fluid retention, careful observation is required when conditions that might be influenced by this factor are present (e.g., asthma, epilepsy, migraine, and cardiac or renal dysfunction).
3. Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as uterine bleeding, mastodynia, etc.
4. Prolonged administration of unopposed estrogen therapy has been reported to increase the risk of endometrial hyperplasia in some patients. Estrogens should be used with caution in patients who have or have had endometriosis.
5. Oral contraceptives appear to be associated with an increased incidence of mental depression. Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.
6. Preexisting uterine leiomyomata may increase in size during prolonged estrogen use. If this occurs, estrogen therapy should be discontinued while the cause is investigated.
7. In patients with a history of jaundice during pregnancy, there is an increased risk that jaundice will recur with the use of estrogen-containing oral contraceptives. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated.
8. Estrogens may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients.
9. Because the prolonged use of estrogens influences the metabolism of calcium and phosphorus, estrogens should be used with caution in patients with metabolic bone diseases associated with hypercalcemia and in patients with renal insufficiency.

Drug/Laboratory Test Interactions

The results of certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The following changes have been observed with large doses of oral estrogen:

1. increased sulfobromophthalein retention;
2. increased prothrombin time; increased factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability;
3. increased thyroxine-binding globulin (TBG), leading to increased circulating total thyroid hormone (T_4) as measured by column or radioimmunoassay; free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered; TBG was not affected in clinical trials of Estraderm;
4. reduced response to the metyrapone test;
5. reduced serum folate concentration;
6. increased serum triglyceride and phospholipid concentration, and decreased pregnanediol excretion.

The pathologist should be informed that the patient is receiving estrogen therapy when relevant specimens are submitted.

Carcinogenesis, Mutagenesis, Impairment of Fertility

See **WARNINGS** and Boxed Warning.

Long-term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver.

Pregnancy Category X

See **CONTRAINDICATIONS** and Boxed Warning.

Estrogens should not be used during pregnancy.

Nursing Mothers

As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS

See **WARNINGS** and Boxed Warning regarding potential adverse effects on the fetus, induction of malignant neoplasms, increased incidence of gallbladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.

The most commonly reported adverse reaction to Estraderm in clinical trials was redness and irritation at the application site. This occurred in about 17% of the women treated and caused approximately 2% to discontinue therapy.

The following additional adverse reactions have been reported with estrogenic therapy, including oral contraceptives:

Genitourinary System: Breakthrough bleeding, spotting, change in menstrual flow; increase in size of uterine fibromyomata; change in cervical erosion and amount of cervical secretion.

Endocrine: Breast tenderness, breast enlargement.

Gastrointestinal: Nausea, vomiting; abdominal cramps, bloating; cholestatic jaundice have been observed with oral estrogen therapy.

Eyes: Steepening of corneal curvature; intolerance to contact lenses.

Central Nervous System: Headache, migraine, dizziness.

Miscellaneous: Change in weight, edema, change in libido.

Do not store above 86°F (30°C).

Do not store unpouched. Apply immediately upon removal from the protective pouch.

CAUTION: Federal law prohibits dispensing without prescription.

C I B A

CIBA Pharmaceutical Company, Division of CIBA-GEIGY Corporation
Summit, New Jersey 07901

© 1986, CIBA.

Treatment of menopausal hot flashes with transdermal administration of clonidine

Manubai Nagamani, M.D., Mae Ellen Kelver, M.D., and Edward R. Smith, Ph.D.
Galveston, Texas

A randomized prospective double-blind study was performed to evaluate the efficacy of a transdermal therapeutic system delivering clonidine in the treatment of menopausal hot flashes. Frequency, severity and duration of the flushing attacks before and during the 8-week treatment period were evaluated. The reduction in the number of hot flashes was highly significant in patients receiving the clonidine transdermal therapeutic system. On subjective comparison of flushing attacks before and during treatment, of the 15 patients who received the clonidine transdermal therapeutic system, 80% reported fewer hot flashes; 73% a decrease in severity; and 67% a decrease in duration. Among the 14 patients who were treated with placebo only, 36% reported fewer hot flashes; 29% a decrease in severity; and 21%, shorter duration (frequency, $p < 0.04$; severity, $p < 0.04$; and duration, $p < 0.03$). Reported side effects were minimal, and no significant effect was observed on blood pressure or pulse rate. Transdermal clonidine therapy had no effect on the pulsatile luteinizing hormone secretion. (Am J Obstet Gynecol 1987;156:561-5.)

Key words: Clonidine, transdermal therapy, hot flashes, menopause

Hot flashes are the most annoying symptoms of menopause. They are experienced by 75% to 85% of women going through natural menopause and 37% of premenopausal women undergoing oophorectomy.[1, 2] Estrogen replacement therapy is the most effective form of treatment for relief of menopausal hot flashes. However, when the patient has contraindications to estrogen, alternate forms of therapy become essential. Even though the exact pathophysiologic mechanism of hot flashes is not known, it has been speculated that it is related to catecholamine excess, since similar symptoms are observed in conditions such as pheochromocytoma that are accompanied by catecholamine excess.[3] Casper et al.,[4] however, measured plasma concentrations of catecholamines during episodes of hot flashes and found no such increase and concluded that an excess of central adrenergic activity is probably responsible for the onset of a hot flash. Clonidine, an α-adrenergic agonist, when given orally, has been shown to reduce the frequency of hot flashes.[5, 6]

The present study was undertaken to evaluate the effectiveness of clonidine when given by a transdermal therapeutic system in the treatment of menopausal hot flashes.

From the Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, University of Texas Medical Branch.
Supported in part by a grant from Boehringer Ingleheim, Ltd.
Received for publication May 20, 1986; revised August 20, 1986; accepted September 26, 1986.
Reprint requests: Manubai Nagamani, M.D., Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX 77550.

Material and methods

Thirty women, six who had undergone spontaneous menopause and 24 who had undergone surgical removal of the ovaries, were included in the study. The mean age of the patients was 41 years (range 25 to 58 years). The frequency of hot flash episodes ranged from 10 to 100 per week. All the patients had stopped estrogen or any other treatment for hot flashes at least 30 days before inclusion in the study. Patients with a history of previous treatment with oral clonidine or a history of hepatic, cardiac, or renal disease or skin diseases of any kind were excluded from the study. The study was conducted in a double-blind fashion. The patients were assigned prospectively to receive either the clonidine transdermal therapeutic system (Dixarit-TTS) or placebo. The placebo and clonidine systems were alike in appearance and neither the physician nor the patient knew who was receiving the therapeutic agent. The systems were provided by Boeringer Ingelheim, Ltd., Ridgefield, Connecticut. The transdermal therapeutic system has the appearance of a small (3.5 cm^2) adhesive bandage. It is composed of a four-layer film, 0.2 mm thick, consisting of a backing layer of pigmented polyester film, a drug reservoir of clonidine, a microporous polypropylene membrane that controls the rate of delivery, and a layer of adhesive formulation of clonidine. Before use, a protective peel strip of polyester that covers the adhesive layer is removed. After application of the system to the intact skin, clonidine in the adhesive layer saturates the skin sites below the system. Clonidine from the drug reservoir then begins to flow through the rate-controlling

Table I. Effect of treatment with the clonidine transdermal therapeutic system and placebo on numbers of hot flashes

	Clonidine							
	Before treatment	*Weeks of treatment*						
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Mean	41	25	19	20	20	16	14	14	12
SD	28	19	17	19	14	20	20	20	17
p Value		0.02	0.001	0.01	0.03	0.02	0.01	0.01	0.002

Table II. Subjective evaluation by patients of frequency, severity, and duration of hot flashes

	Frequency		*Severity*		*Duration*	
	Clonidine	*Placebo*	*Clonidine*	*Placebo*	*Clonidine*	*Placebo*
Decrease	12*	5	11*	4	10†	3
Same	3	9	4	10	5	11

*$p < 0.04$.
†$p < 0.03$.

membrane and the adhesive layer of the system. The agent is absorbed from the devise through the skin, at a rate of 0.1 mg/day, and is designed to maintain steady-state plasma levels of clonidine for 7 days.

The systems were applied to the upper outer arm and were changed once a week. The patients participated in the study for 10 weeks, 2 weeks without treatment to obtain baseline values and 8 weeks with treatment. Each patient kept a diary and recorded the occurrence of hot flashes and the side effects of treatment during the treatment period. During their visits and at the end of the study, the patients were questioned about the frequency, severity, and duration of hot flashes. Evaluation by the patients of the severity and duration of hot flashes during treatment was purely subjective and no method of quantification was used. The patients were seen at weekly intervals and the blood pressure and pulse rate were recorded at each visit. The transdermal therapeutic system was also changed at these visits.

Fifteen patients received the clonidine transdermal therapeutic system and 15, placebo. One of the patients receiving placebo had dropped out after 2 weeks of treatment and this patient was excluded from statistical analysis.

Hormone analysis. Two of the patients who completed the study and had complete elimination of hot flashes with clonidine transdermal therapeutic system treatment were subsequently studied with frequent sampling of blood for measurement of luteinizing hormone levels. Samples of blood were obtained at 15-minute intervals during a 6-hour period without treatment when these patients experienced 88 and 76 hot flashes per week, respectively, and 2 weeks after application of the clonidine transdermal therapeutic system when they had no hot flashes. The luteinizing hormone levels were measured by double-antibody radioimmunoassay with the materials supplied by the hormone distribution program of the National Institute of Arthritis, Metabolism, and Digestive Diseases and the results are expressed as milli–International Units, with reference to the second international preparation of human menopausal gonadotropins.

Statistical analysis was done by the χ^2 method. The mean numbers of hot flashes before and during treatment were compared by two-way analysis of variance.

Results

The mean number of hot flashes before and during treatment in patients receiving clonidine and placebo are shown in Table I. Even though there was some decrease in the number of hot flashes in women treated with placebo, the reduction in the number of hot flashes was highly significant in patients receiving clonidine. The number of hot flashes recorded as a baseline before treatment was compared with the number recorded each week during the study. The mean decrease for each week, relative to the baseline, is shown in Fig. 1. On subjective comparison of flushing attacks before and during treatment, 12 of 15 patients (80%) who were receiving the clonidine transdermal therapeutic system reported a reduction in the frequency of hot flashes during treatment while only five of 14 patients (36%) receiving placebo reported a reduction ($p < 0.04$ Table II). Of the 12 patients receiving clonidine who reported a decrease in the number of hot flashes, four

	Placebo							
	Weeks of treatment							
Before treatment	1	2	3	4	5	6	7	8
36	30	28	29	27	25	24	25	20
20	19	18	20	18	18	17	27	18
	NS	NS	NS	NS	0.05	0.05	NS	0.04

were completely without hot flashes at the end of 8 weeks and the other eight had a reduction in the number of hot flashes of ≤60%. Eleven of 15 patients (73%) who were receiving the clonidine transdermal therapeutic system reported a decrease in the severity of hot flashes while only four of 14 patients receiving placebo (29%) reported a decrease ($p < 0.04$). The duration of flushing attacks decreased in 10 of 15 patients who received clonidine (67%) but in only three of 14 patients who received placebo ($p < 0.03$). Side effects were minimal and of the same character for both groups. Transient local skin reactions (erythema and/or itching) were observed in four patients using the clonidine transdermal therapeutic system and three receiving placebo. None of the patients receiving the clonidine transdermal therapeutic system complained of dryness of the mouth or drowsiness, which are the common side effects reported with oral clonidine therapy.[5] There were no significant changes in the blood pressure or pulse rate during the study period.

Even though clonidine abolished the hot flashes, it did not abolish the pulsatile secretion of luteinizing hormone. Luteinizing hormone pulses (increment from nadir to peak of >20%) were present during treatment with the clonidine transdermal therapeutic system and the number of luteinizing hormone pulses observed during the 6-hour period during treatment was about the same as that before treatment (Fig. 2). Coefficient of variation of luteinizing hormone levels during the 6-hour period during treatment with clonidine was similar to that before treatment (14% before and 19% during treatment in Patient No. 1 and 22% before and 23% during treatment in Patient No. 2).

Comment

Clonidine is an imidazoline derivative that has been available in an oral form for treatment of hypertension since 1974. An oral preparation of clonidine has also been used in the treatment of hot flashes.[5,6] The dosage that was used in these studies for the treatment of menopausal flushing is lower than that used for hypertension. In the present study clonidine administered transdermally was effective in reducing the incidence, severity, and duration of hot flashes. Possible advantages of transdermal therapeutic system therapy over the oral treatment includes: (1) its effectiveness for 7 days after application, since once-a-week application is more convenient to the patient, and (2) its reduced drug input into the body.

All previous studies on clonidine for treatment of menopausal hot flashes have been with oral therapy. Clayden et al.,[5] in a multicenter, placebo-controlled, double-blind crossover study, observed that clonidine had a statistically high significant effect in controlling menopausal hot flashes. Edington et al.,[6] in a similar study, reported that clonidine significantly reduced the frequency of hot flashes even though the placebo effect was substantial. In a study restricted to patients who had undergone surgical castration. Ylikorkala[7] determined that clonidine significantly dimished the frequency of hot flashes. Schindler et al.[8] observed a significant decrease in the number of hot flashes with clonidine administered orally at a dosage of 150 μg daily. In contrast to these reports, Salmi and Punnonen[9] reported that the effect of clonidine was not significantly different from that of the placebo. Lack of effectiveness in this study could have been due to a dose of <100 μg used by some of the patients in the study. Bolli and Simpson[10] performed a double-blind study in postmenopausal women who were being treated with clonidine for hypertension and determined that clonidine at a dosage of 150 μg daily significantly reduced the menopausal flushing when compared with placebo, while at a dose of 75 μg daily the effect was not greater than that of placebo. The dosage that was delivered by the clonidine transdermal therapeutic system in our present study was 100 μg daily. The placebo effect that was observed in the present study and the previous studies with oral clonidine therapy indicates that emotional and psychological influences accompany the physiologic changes of menopause.

Hot flashes have been demonstrated to precede pulsatile release of luteinizing hormone from the pituitary.[4] The theoretical basis for the use of clonidine for treatment of hot flashes lies in the assumption that the symptoms are triggered by a hypothalamic mechanism that is involved with the pulsatile release of gonadotropin-releasing hormone.[4] Although the exact mech-

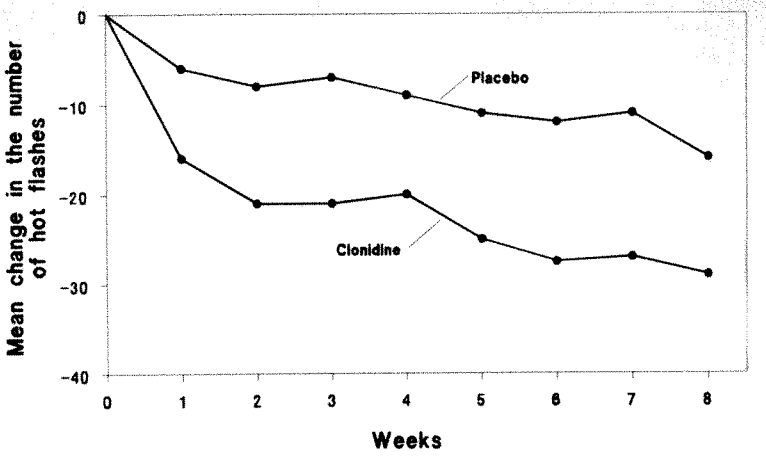


Fig. 1. Mean decrease in the number of hot flashes from initial values.

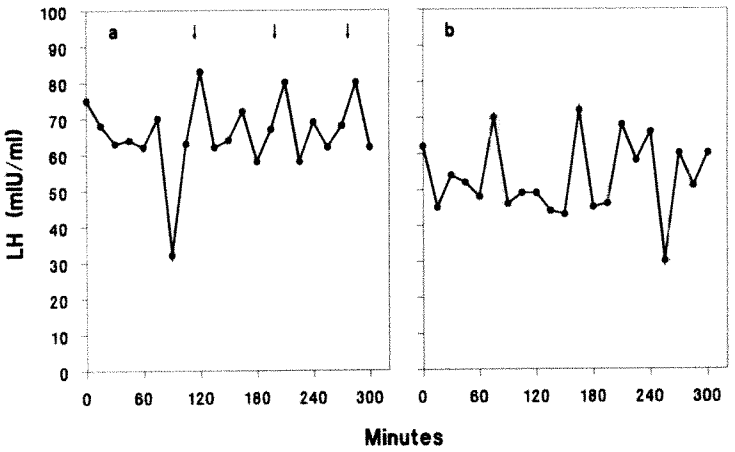


Fig. 2. Serum luteinizing hormone *(LH)* concentrations in a menopausal patient before *(a)* and during *(b)* treatment with the clonidine transdermal therapeutic system. *Arrows* indicate onset of hot flashes.

anism by which clonidine relieves hot flashes is not known, the effect of clonidine on hot flashes appears not to be determined centrally since the hot flashes were eliminated without any change in the pulsatile secretion of luteinizing hormone. Tulandi et al.[11] observed no changes in the number of luteinizing hormone pulses or the total luteinizing hormone secretion after intravenous clonidine administration. Schindler et al.[8] reported no changes in plasma estradiol, luteinizing hormone, follicle-stimulating hormone or prolactin levels in menopausal women receiving oral clonidine for treatment of hot flashes. In earlier studies in animals, clonidine was found to reduce the peripheral vascular responsiveness to vasodilator and vasconstrictor stimuli.[12, 13] Reduction of peripheral vascular reactivity might be one of the mechanisms by which clonidine relieves hot flashes.

In summary, clonidine transdermal therapeutic system therapy would be ideal for symptomatic treatment of hot flashes in postmenopausal women who have contraindications to estrogen replacement therapy. Since the transdermal therapeutic system is effective for 7 days, once-a-week application is more convenient to the patient.

REFERENCES

1. Mulley G, Mitchell JRA. Menopausal flushing: does oestrogen therapy make sense? Lancet 1976;1:1397-8.
2. Aksel S, Schomberg DW, Tyrey L, Hammond CB. Vasomotor symptoms, serum estrogens, and gonadotropin levels in surgical menopause. Am J Obstet Gynecol 1976;126:165-9.
3. Hickler RB, Thorn GW. Pheochromocytoma. In: Thorn GW, Adams RD, Braunwald E, Isselbacher K, Petersdorf RG, eds: Harrison's principles of internal medicine. New York: McGraw-Hill, 1977:557-63.
4. Casper RF, Yen SSC, Wilkes MM. Menopausal flushes: a neuroendocrine link with pulsatile luteinizing hormone secretion. Science 1979;205:823-5.

5. Clayden JR, Bell JW, Pollard P. Menopausal flushing: double-blind trial of a non-hormonal medication. Br Med J 1974;1:409-12.
6. Edington RF, Chagnon JP, Steinberg WM. Clonidine (Dixarit) for menopausal flushing. Can Med Assoc J 1980; 123:23-6.
7. Ylikorkala O. Clonidine in the treatment of menopausal symptoms. Ann Chir Gynaecol Fenn 1975;64:242-5.
8. Schindler AE, Muller D, Keller E, Goser R, Runkel F. Studies with clonidine (Dixarit) in menopausal women. Arch Gynecol 1979;227:341-7.
9. Salmi T, Punnonen R. Clonidine in the treatment of menopausal symptoms. Int J Gynaecol Obstet 1979; 16:422-6.
10. Bolli P, Simpson FO. Clonidine in menopausal flushing: a double-blind trial. Aust NZ Med J 1975;82:196-7.
11. Tulandi T, Lal S, Kinch RA. Effect of intravenous clonidine on menopausal flusing and luteinizing hormone secretion. Br J Obstet Gynaecol 1983;90:854-7.
12. Gaskel P, Melnyk J. The effect of clonidine on vascular reactivity to anquiotensin, noradrenaline and vasopressin in conscious rats. Can J Physiol Pharmacol 1977;56:23-9.
13. Zaimis E, Hanington E. A possible pharmacological approach to migraine. Lancet 1969:2:298-300.

Vaginal birth after two or more previous cesarean sections

George Farmakides, M.D., Roger Duvivier, M.D., Harold Schulman, M.D., Elizabeth Schneider, M.D., and John Biordi, M.D.

Mineola, New York

The recommendation from the American College of Obstetricians and Gynecologists is to allow vaginal delivery after one cesarean section. This report is an update of our experience of 57 women with two or more cesarean sections who were allowed to labor. (Am J Obstet Gynecol 1987;156:565-6.)

Key words: Previous cesarean section, labor, outcome

Recently, the American College of Obstetricians and Gynecologists recommended that women with one previous cesarean section be allowed a trial of labor. One of the exclusions to this recommendation was women with more than one previous cesarean section. Presumably this recommendation was based on the belief that there were not sufficient data to make such a prescription. Unfortunately, there is also no data base to suggest that repeat cesarean section is necessary in these women. This report updates our continuing experience with allowing women to labor after more than one previous cesarean section.

Material and methods

Women in this report were cared for at the Bronx Municipal Hospital of the Albert Einstein College of Medicine and the Winthrop-University Hospital. It has been our policy since 1978 to offer a trial of labor to all women with previous cesarean section unless there was a medical or obstetric complication that contraindicated labor.[1] We considered a vertical uterine incision to be a contraindication. There were 57 women who experienced at least 4 hours of labor (39 with two and 18 with three previous cesarean sections). For evaluation of comparative morbidity we selected 64 women during the same time period who had repeat operations without labor.

Table I. Outcomes in women with more than two previous cesarean sections related to primary indication

Primary indication	*Cesarean section in labor*	*Vaginal delivery*	*Cesarean section without labor*
Dystocia	7	16	40
Malpresentation	1	10	4
Abruptio placentae, toxemia, twins	—	4	3
Fetal distress	2	6	14
Uncertain	3	8	3
Total	13	44	64

In the study group n = 121: no labor, 64; labor, 57; vaginal birth, 44 (77%).

Results

Table I summarizes the outcomes in relationship to the primary indication. Forty-four or 77% of those given a trial of labor were successfully delivered. This finding was not affected by the primary indication for the first cesarean section. There were no differences in neonatal weight among the three groups. There was

From the Departments of Obstetrics and Gynecology, Winthrop-University Hospital.
Received for publication September 3, 1986; accepted September 10, 1986.
Reprint requests: George Farmakides, M.D., 259 First St., Mineola, NY 11501.

no significant perinatal morbidity. Oxytocin was used intravenously in five women, the indications being arrest of labor and premature rupture of the membranes. A uterine scar defect was noted in one woman who was delivered vaginally. There were no observable problems associated with this silent dehiscence. There was no febrile morbidity in the vaginal delivery group. There were three women who became febrile when there was labor with subsequent cesarean section or when cesarean section was done electively. Eighty percent of the women undergoing cesarean section received antibiotics, but none who were delivered vaginally did so. The average length of hospital stay was 3 days for vaginal births and 6 days with cesarean section.

Comment

This report adds modest numbers to those already reported, which seem to indicate that labor is safe after more than one cesarean section. It appears that if there is risk in allowing labor, it must be small or even less than that associated with repeat cesarean section. We hope that others will also give women the opportunity to labor under the safety guidelines recommended by the ACOG so that large enough numbers can be achieved to demonstrate the safety or danger of this approach.

REFERENCES

1. Saldana L, Schulman H, Reuss L. Management of pregnancy after cesarean section. AM J OBSTET GYNECOL 1979;135:555-9.
2. Porreco RP, Meier PR. Trials of labor in patients with multiple previous cesarean sections. J Reprod Med 1983; 28:770-2.
3. Paul RH, Phelan JP, Yeh S. Trial of labor in the patient with a prior cesarean birth. AM J OBSTET GYNECOL 1985;151:297-304.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1987 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 156 (January-June) and Vol. 157 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Prospective pricing system by diagnosis-related groups: Comparison of federal diagnosis-related groups with high-risk obstetric care groups

Michael B. Resnick, Ed.D., Mario Ariet, Ph.D., Randolph L. Carter, Ph.D., Andres Cao, M.B.A., Robert R. Furlough, Ph.D., Janet H. Evans, M.S.P.A., Allan G. W. McLeod, M.D., Amelia C. Cruz, M.D., Richard L. Bucciarelli, M.D., John S. Curran, M.D., and William W. Ausbon, M.D.

Gainesville, Tallahassee, Miami, and Tampa, Florida

Of 468 diagnosis-related groups identified by the federal government for Medicaid reimbursement, 15 are related to obstetric hospital care. Each diagnosis-related group is considered a distinct group in which cases are homogeneous with respect to resource consumption. Because the diagnosis-related group system is based primarily on data from community and secondary care hospitals, it does not differentiate sufficiently among high-risk obstetric patients seen at tertiary care institutions, such as Florida's Regional Perinatal Intensive Care Centers. We developed an alternative scheme for diagnosis-related groups, called obstetric care groups, using the federal diagnosis-related groups as the model from which to depart. Data collected for 4192 women during a 2½-year period indicate that obstetric care groups provide more homogeneous groups than diagnosis-related groups for our population of high-risk patients. The obstetric care groups differentiate between no complications, one complication, and two or more complications, while the diagnosis-related groups differentiate only between no complications and one or more complications. Also, complications for obstetric care groups are based on only 19 diagnoses that contribute significantly to resource consumption, while the list of possible complications exceeds 200 for diagnosis-related groups. Although the obstetric care group classification system is simpler than that for diagnosis-related groups, it results in a more accurate reimbursement of hospitalization charges for high-risk obstetric care. (Am J Obstet Gynecol 1987;156:567-73.)

Key words: Prospective pricing system, diagnosis-related groups, Medicaid reimbursement, high-risk obstetric hospitalization

In 1975, a group of investigators at Yale University developed an innovative approach to reimbursement for hospitalization, termed diagnosis-related groups (DRGs).[1, 2] Basically, they proposed a prospective pricing system of hospital charges, rather than the traditional retrospective pricing system in which hospital charges are calculated by totaling daily room charges plus specific goods and services consumed. Later adopted by the State of New Jersey, this prospective pricing system made provision for a fixed amount per discharge, based primarily on the patient's diagnosis, not on the number of days hospitalized and services utilized.[3] Each DRG was considered a distinct group in which cases were homogeneous with respect to resource consumption.[4, 5]

At the federal level, the Health Care Financing Administration adopted DRGs for Medicare reimbursement in 1983, stating that this prospective pricing system would help establish the federal government as a "prudent buyer" of hospital services.[6] Peer review, they maintained, would continue to ensure appropriateness of admissions and discharges and overall quality of care. Under the traditional method of reimbursement, there were few or no restrictions placed on cost of care.[7] In contrast, the new prospective pricing system would reimburse a standard payment for an "average" patient in a given DRG category, regardless of actual costs incurred. The rationale was that all patients within the same DRG classification could be expected to have similar medical requirements; therefore hospital gains and losses would average out, so that in the long run hospital costs would equal reimbursements.[8-10] The DRG

From the College of Medicine and the Department of Statistics, University of Florida, Gainesville, the Children's Medical Services Program, State of Florida, Tallahassee, the University of Miami College of Medicine, Miami, and the University of South Florida College of Medicine, Tampa.

This project was funded in part under an agreement with the Department of Health and Rehabilitative Services, State of Florida, Children's Medical Services.

Received for publication September 4, 1986; accepted October 29, 1986.

Reprint requests: Michael B. Resnick, Box J-296, JHMHC, Department of Pediatrics, University of Florida College of Medicine, Gainesville, FL 32610.

Table I. Federal obstetric DRGs

Group No.	*Diagnosis*	*DRG weight*	*Average length of stay* (days)*	*Outlier cutoff (days)*	*Outlier weight*
370	Cesarean section with complications	0.9912	7.2	15	0.083
371	Cesarean section without complications	0.7535	6.1	10	0.074
372	Vaginal delivery with complications	0.5534	3.8	9	0.087
373	Vaginal delivery without complications	0.4063	3.2	9	0.076
374	Vaginal delivery without complications, but with sterilization and/or dilatation and curettage	0.5492	3.6	7	0.092
375	Vaginal delivery with surgery except sterilization or dilatation and curettage	0.6889	4.4	15	0.094
376	Postpartum diagnosis without operation	0.4158	2.9	15	0.086
377	Postpartum diagnosis with surgery	0.4761	2.2	8	0.130
378	Ectopic pregnancy	0.8094	5.5	11	0.088
379	Threatened abortion	0.3169	2.2	8	0.086
380	Abortion without dilatation and curettage	0.2705	1.5	4	0.108
381	Abortion with dilatation and curettage	0.3602	1.4	4	0.154
382	False labor	0.1842	1.2	2	0.092
383	Other antepartum diagnosis with complications	0.4317	3.4	14	0.076
384	Other antepartum diagnosis without complications	0.3245	2.2	9	0.088

*Geometric average length of stay, rather than arithmetic average, was calculated as a more accurate reflection of central tendency.

system was expected to discourage hospitals from lengthening hospital stays unnecessarily and providing goods and services not clearly required.

In Florida, measures for cost containment were taking directions similar to those on the federal level. For example, the Health Care Consumer Protection Act of 1984 placed several constraints on hospitals in an effort to control escalating costs.[11] Proviso language in the 1984 Appropriations Act mandated the development of a prospective pricing system for neonatal intensive care services provided by the 10 Florida Regional Perinatal Intensive Care Centers. These centers give obstetric care to high-risk pregnant women, primarily low-income, and neonatal intensive care to sick or low birth weight infants throughout the state.[12] The federal DRG system did not differentiate enough among expensive neonatal hospitalizations of these infants according to severity of illness, and it did not reimburse adequately. The state needed its own fiscal management tool for determining equitable reimbursement. Children's Medical Services, the state agency that funds the care of this population of infants, requested an analysis of the medical and fiscal data from the records of all neonates cared for in the participating hospitals between July 1, 1980, and June 30, 1985 (8492 infants). As a result of this analysis, a new DRG-type classification system, called neonatal care groups, was developed, providing more equitable reimbursement.[13] This method was implemented July 1, 1985.

A similar type of discrepancy (noted above, between federal DRGs and Florida's experience with neonatal intensive care) was thought to exist between the population sampled to develop the federal obstetric DRGs and our population of high-risk obstetric patients; therefore the current study was undertaken. Furthermore, proviso language in the 1985 Appropriations Act mandated the development of a prospective pricing system for high-risk obstetric care. Hospital charges, diagnoses, procedures, length of hospital stay, and other clinical and fiscal variables had been accumulated for high-risk obstetric patients in the Children's Medical Services statewide data system.[14] These data were used to develop a valid pricing system for high-risk obstetric care.

In this report, we describe the federal DRG system as it applies to obstetric care and the development and applications of Florida's high-risk obstetric care groups (OBCGs).

Federal DRG system

The federal DRG system uses 468 categories, of which 15 pertain to obstetric care (Table I). The major divisions are antepartum, intrapartum, and postpartum hospitalizations. Within the intrapartum type, a further main division is based on whether delivery is cesarean or vaginal. The other major distinctions among groups are whether complications are present and whether operation is performed. Complications are defined as the presence of one or more of a list of more than 200 diagnoses considered in the DRG system to be potentially related to pregnancy.

DRG "weight" is a concept used in the federal system to indicate the intensity and duration of hospital care (and hence the cost) for an average patient in a given group. Weights are based on geometric average charges (a more accurate reflection of central tendency than arithmetic average). The lowest DRG weight is 0.182, for antepartum admission due to false labor (DRG 382).

Table II. Complications that significantly predict hospital charges in high-risk obstetric patients

	Estimated effect	*Frequency of occurrence (%)*	*Partial R^2*	*No. of complications*
Antepartum complication				
Kidney disease	0.51	1.7	0.0076	1
Hyperemesis with ketonuria	0.69	0.8	0.0132	1
Pulmonary dysfunction	0.54	2.3	0.0159	1
Spontaneous premature labor	0.22	25.9	0.0171	1
Viral infection	0.50	1.3	0.0079	1
Amnionitis	0.41	3.0	0.0054	1
Maternal diabetes	0.32	16.7	0.0200	1
Intrapartum complication				
Psychological disorder	0.20	0.8	0.001	1
Hypertension	0.08	7.7	0.001	0
Collagen vascular disease	0.26	0.5	0.0007	1
Amnionitis	0.25	3.0	0.01	1
Arrested progressive labor	0.11	2.7	0.001	0
Polyhydramnios	0.14	1.3	0.0006	0
Premature birth	0.05	23.3	0.007	0
Dilatation and curettage	0.40	1.0	0.0015	1
Endometritis	0.21	2.6	0.003	1
Hemorrhage	0.22	0.9	0.0012	1
Maternal death	1.01	0.2	0.003	2
Tubal ligation	0.25	14.5	0.015	1
Hysterectomy	0.60	0.4	0.005	2
X-ray film obtained	0.15	3.4	0.002	0
Prematurely ruptured membranes	0.11	17.4	0.006	0
Delivery type	0.68	37.1	0.40	—
Maternal diabetes	0.20	16.7	0.014	1
Eclampsia	0.34	0.8	0.0024	1
Fetal monitoring	0.10	72.0	0.004	0
Preeclampsia	0.32	13.2	0.03	1

The lowest intrapartum (delivery) DRG weight is 0.4063, for vaginal delivery without complications (DRG 373). The highest overall DRG weight is 0.9912, for cesarean section with complications (DRG 370). To calculate reimbursement, the DRG weight is multiplied by a "base rate," currently about $3000. For example, reimbursement for a woman having a vaginal delivery with no complications would be: DRG weight × base rate = reimbursement, or 0.4063 × $3000 = $1219. By this method, DRG weights can remain stable, as they are relative and not absolute numbers. The base rate fluctuates with the economy and as a reflection of the current cost of doing business.

"Outlier cutoff" is a concept used to reimburse hospitals for patients with unusually long, expensive hospitalizations.[4] The outlier cutoff is the day of hospitalization considered to lie significantly beyond the geometric average length of stay, for which additional reimbursement is warranted. For example, the geometric average length of stay for a vaginal delivery without complications (DRG 373) is 3.2 days, and the outlier cutoff is 9 days. If an obstetric patient in this DRG is hospitalized more than 9 days, the hospital is reimbursed an additional sum for every outlier day, according to an "outlier weight." At an outlier weight of 0.076 for uncomplicated vaginal delivery, the sum for a woman staying for 12 days would be calculated by the equation: Outlier weight × outlier days × base rate = additional reimbursement, or 0.076 × 3 × $3000 = $684. DRG categories, weights, and outlier cutoff days are shown in Table I.

The federal prospective pricing system with the use of DRGs has been adopted as the basis of Medicaid payment in nine states: New Jersey, Michigan, Ohio, Pennsylvania, Utah, Washington, Minnesota, Oregon, and South Dakota.[13] Blue Cross has implemented DRG-based reimbursements in Arizona, Kansas, Michigan, Nebraska, and Oklahoma.[15, 16]

Obstetric patients and hospital charges in Florida's Regional Perinatal Intensive Care Centers

The records of all sponsored obstetric patients who were hospitalized for obstetric care in Florida's Regional Perinatal Intensive Care Centers between July 1, 1983, and December 31, 1985, were analyzed for fiscal and medical data in a total of 4192 women with hospital charges of $17.47 million. This was possible because demographic, medical, and fiscal data for each mother had been collected, coded, and entered into the data system of the Regional Perinatal Intensive Care Center since 1980.[14] To qualify for participation in the program, each woman had to be classified as "high risk" by her local physician or a screening clinic and referred

Table III. Florida OBCGs

Group No.	*Description*	*OBCG weight**	*Average length of stay† (days)*	*Outlier cutoff day‡*	*Outlier weight*
370.0	Cesarean section, 0 complication	3.0	6.5	10	0.23
370.1	Cesarean section, 1 complication	3.2	7.2	15	0.22
370.2	Cesarean section, ≥2 complications	3.9	8.0	15	0.24
372.0	Vaginal delivery, 0 complication	1.3	3.3	8	0.20
372.1	Vaginal delivery, 1 complication	1.8	4.4	10	0.20
372.2	Vaginal delivery, ≥2 complications	2.2	4.9	10	0.22
376.0	Postpartum	1.5	4.8	15	0.16
383.0	Antepartum, 0 complication	1.3	4.3	10	0.15
383.1	Antepartum, 1 complication	1.7	5.5	15	0.15
383.2	Antepartum, ≥2 complications	2.3	6.9	15	0.17

*Weight reflects both intensity of care and length of hospitalization. Base rate (currently $1000 in Florida) is multiplied by weight to determine total reimbursement for hospitalization. Example: For an uncomplicated vaginal delivery, reimbursement would be: 1.3 × $1000 = $1300.

†Calculated as geometric average length of stay, which reflects central tendency more accurately than arithmetic average.

‡Outlier cutoff day is the day of hospitalization considered to be the limit of a typical hospital stay, beyond which additional reimbursement is allowed. To calculate this additional reimbursement, the base rate of $1000 is multiplied by the appropriate outlier weight, and the result is multiplied by the number of hospitalization days beyond the outlier cutoff day. Example: Reimbursement for a woman hospitalized 18 days for Cesarean section delivery with one complication would be $3200 for the first 15 days (3.2 × $1000) plus 3 days beyond outlier cutoff. Outlier days would be calculated by: days × base rate × outlier weight, or 3 × $1000 × 0.22 = $660. Thus total reimbursement would be $3860.

for tertiary care to one of the 10 Regional Perinatal Intensive Care Centers. She also had to meet the low-income criteria for sponsorship in the program. During the 2½-year study period, 3784 of the 4192 obstetric patients admitted to the program were delivered in a Regional Perinatal Intensive Care Center; the remaining 408 came only for prenatal care and were delivered elsewhere or had a spontaneous abortion.

Hospitalizations and charges were clustered into three main groups: antepartum (predelivery), intrapartum (delivery), and postpartum. If a patient had several antepartum hospitalizations, charges for these hospitalizations were totaled and treated as one hospitalization. Similarly, multiple postpartum hospitalizations were combined.

With the above definitions, the 4192 obstetric patients had 1303 antepartum hospitalizations, 3784 intrapartum hospitalizations, and 88 postpartum hospitalizations. About one third of the women had antepartum hospitalizations. Not many patients had catastrophic charges. Only 23 women had hospital bills exceeding $20,000, and the highest individual charge was $83,000. Of the eight patients who died, five were in the group having charges above $20,000.

Development of Florida's OBCGs

Analysis of the data for the Regional Perinatal Intensive Care Center patients suggested that the federal classifications of antepartum, intrapartum, and postpartum hospitalizations were warranted for our population of high-risk obstetric patients. However, the extensive list of diagnoses used in the federal system to define complications appeared to be unnecessary. Therefore we identified approximately 150 variables contained in the data system of the Regional Perinatal Intensive Care Centers that were judged to have potential for significant fiscal impact. When these variables were analyzed by stepwise regression to determine their effects on hospital charges, 27 emerged as significant (Table II), seven antepartum and 20 intrapartum. Stepwise regression analyses were performed separately for antepartum and intrapartum hospitalizations. Postpartum diagnoses composed only 1.7% of all admissions and were not analyzed by stepwise regression, since this group could have little overall fiscal impact in the program.

Regression analyses demonstrated that delivery type (vaginal or cesarean) was the variable most predictive of hospital charges, accounting for 0.376 of the variance in log charges (out of a total of 0.54 with the use of all variables). Therefore we divided the category "intrapartum" into "vaginal delivery" and "cesarean section." No similar subdivision on the basis of a single predictor variable appeared justified for antepartum and postpartum categories.

The fact that no single variable in any hospitalization category, other than delivery type, was a dominant predictor of hospital charges suggested another approach to defining diagnoses and conditions that would predict charges. "Estimated effects" (coefficients) were calculated for all variables to indicate their individual contributions to charges (Table II). The relative magnitudes of the estimated effects were then averaged for each hospitalization type. Each estimated effect was divided by the average effect for that hospitalization type, and the result was then rounded to the nearest whole

Table IV. Comparison of reimbursements between federal obstetric DRGs and Florida OBCGs

Florida OBCGs			*Comparable federal DRGs*		
OBCG No.	*Description*	*Reimbursement*	*DRG No.*	*Description*	*Reimbursement*
370.0	Cesarean section, 0 complication	$3000	371	Cesearan section, 0 complication	$2261
370.1	Cesarean section, 1 complication	$3200	370	Cesarean section, ≥1 complication	$2973
370.2	Cesarean section, ≥2 complications	$3900			
372.0	Vaginal delivery, 0 complication	$1300	373	Vaginal delivery, 0 complication	$1219
372.1	Vaginal delivery, 1 complication	$1800	372	Vaginal delivery, ≥1 complication	$1660
372.2	Vaginal delivery, ≥2 complications	$2200	374	Vaginal delivery, 0 complication, but sterilization and/or dilatation and curretage	$1648
			375	Vaginal delivery with operation but no sterilization or dilatation and curettage	$2067
376.0	Postpartum (all)	$1500	376	Postpartum, no operation	$1247
			377	Postpartum, operation	$1428
383.0	Antepartum, 0 complication	$1300	384	Antepartum, 0 complication	$974
383.1	Antepartum, 1 complication	$1700	378	Ectopic pregnancy	$2428
			379	Threatened abortion	$951
			380	Abortion without dilatation and curettage	$812
			382	False labor	$553
			383	Antepartum, ≥1 complication	$1295
383.2	Antepartum, ≥2 complications	$2300	383	Antepartum, ≥1 complication	$1295
			381	Abortion with dilatation and curettage	$1081

number. With a few exceptions (Table II), this rounded value was taken as the number of complications for which each diagnosis would count.

With this method, six of seven predelivery diagnoses counted as one complication each; the seventh, hyperemesis with ketonuria, had an estimated effect closer to two complications than one, but because it occurred least frequently of all antepartum complications, it was rounded down to one to simplify computations. On the other hand, the estimated effect of spontaneous premature labor divided by the corresponding average was 0.48 and could have been rounded down to zero but was instead rounded up to one complication because of its high frequency.

Of the 20 intrapartum variables, 11 were rounded to one complication, but one of these, x-ray, was changed to zero to discourage unnecessary use of roentgenograms. The other six zeros were for hypertension, arrested progressive labor, polyhydramnios, premature birth, premature ruptured membranes, and fetal monitoring (Table II). The estimated effects of two intrapartum variables were sufficiently high to warrant identifying them as two complications each: maternal death and hysterectomy.

Our analyses indicated that complications should be used additively to define subgroups but that only three subgroups are required. Thus three of our four main OBCGs (370, 372, and 383) were subdivided into no complications, one complication, and two or more complications (suffixes .0, .1, and .2, respectively). Our prefix numbers were chosen to resemble federal DRG numbers as closely as possible. An example of an OBCG classification would be predelivery hospitalization of a diabetic woman with spontaneous premature labor. Premature labor would count as one complication and maternal diabetes as another, putting the patient in OBCG 383.2: antepartum hospitalization with two or more complications. Details of Florida OBCGs appear in Table III.

The computerized management system for patients served at Florida's 10 Regional Perinatal Intensive Care Centers provides a central data base that stores demographic, clinical, and fiscal data for each patient. The system automatically classifies patients into appropriate OBCG sections and computes entitlements. It identifies outlier days (if any), hospital charges, and hospital entitlement. An interactive report, available on line to each participating center, verifies correct entry of each patient's data into the system. The essentials for determining OBCG reimbursement are shown in Table III. The distribution of 5175 obstetric hospitalizations by OBCG category appears in Fig. 1, along with

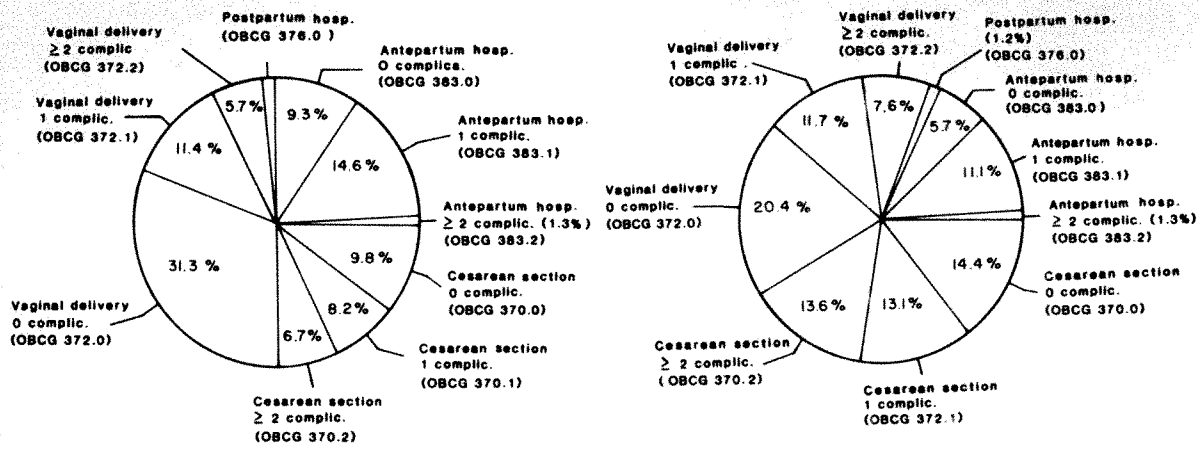


Fig. 1. *Left:* Distribution of 5175 obstetric hospitalizations by Florida OBCG category during the period July 1, 1983, through December 31, 1985. *Right:* Proportion of hospital charges totaling $17.47 million for each Florida OBCG category during the same period.

the proportion of $17.47 million in hospital charges for each category.

Comparison of OBCGs with DRGs

When the federal system of using all variables to define complications for delivery hospitalizations was applied to our data, 40% of the variance in hospital charges could be explained by diagnosis-related groups ($R^2 = 0.4$). When the Florida OBCG system was used, 45.2% of the variance could be explained. This figure is reasonably close to the maximum of 54% that could be obtained with the use of stepwise regression including all of our significant variables (Table II). Thus the simplified OBCG system for delivery hospitalizations predicted log charges more accurately than the corresponding DRG system. Also, the DRG approach uses hundreds of possible diagnoses and conditions to identify "complications," whereas the OBCG system uses only 19 (seven antepartum and 12 intrapartum).

Reimbursements for federal DRGs and Florida's OBCGs are compared in Table IV. Uncomplicated cesarean sections by the OBCG system have charges approximately 25% higher than those of uncomplicated cesarean sections by the DRG system. Cesarean sections with two or more complications in the OBCG system are 30% more expensive than complicated cesarean sections in the DRG system. Also, vaginal deliveries with two or more complications in the OBCG system are reimbursed at a 30% higher rate than complicated vaginal deliveries in the DRG system.

The reimbursement differences between OBCGs and DRGs that occurred in antepartum and postpartum hospitalizations were due to the high-risk nature of the OBCG population compared with the federal population. Moreover OBCGs are based on cumulative antepartum and postpartum time spent in the hospital. Another difference is the method of counting outlier cutoff days; in the OBCG system, outlier cutoff days are counted and reimbursed when all antepartum or intrapartum hospital days added together exceed the outlier cutoff. The OBCG approach of adding length of stays across multiple antepartum (or postpartum) hospitalizations for one patient would tend to discourage long lengths of stay as well as repeated hospitalizations during the same pregnancy.

While outpatient care is not integrated into either the OBCG or the DRG system, it should be noted because outpatient prenatal care is an important component in the treatment of high-risk obstetric patients. The 3784 patients who were delivered in the program had a total of 16,273 outpatient visits and a total of $1.6 million in charges. The average number of prenatal visits was approximately seven for patients who received prenatal care (59% of the total population).

In summary, OBCGs were adopted by Florida's Regional Perinatal Intensive Care Centers program on July 1, 1986. This prospective pricing system has the same advantages as the DRG system over the traditional per diem method as a fiscal management tool. Data collected for 4192 women during a 2½-year period indicate that the system also works better than the federal DRG system for our population of high-risk obstetric patients. The OBCG system results in a manageable number of homogeneous groups that are quickly identified and easy to monitor. The data needed are only whether the hospitalization was for predelivery problems, delivery, or postnatal complications; whether delivery was by cesarean section or vaginal; and whether zero, one, or two complications were present. In addition, only seven antepartum and 12 delivery complications need to be considered, as opposed to hundreds in the DRG system. Although the OBCG in-

trapartum reimbursements for "no complications" and "one complication" are similar to those for the corresponding "uncomplicated" and "complicated" DRG categories, the additional OBCG category for two or more complications provides a significantly higher reimbursement rate for severe problem cases, a distinction that is appropriate for a high-risk obstetric population.

We thank Jim Fletcher, Systems Analyst, and Everton Rowe, Biostatistician, for their technical assistance, and Barbara Cox, Biomedical Editor, for assistance in the preparation of this paper.

REFERENCES

1. Yale School of Organization and Management. The new ICD-9-CM diagnosis-related group classification scheme: definitions of diagnosis related groups. New Haven, Connecticut: Health Systems Management Group, 1981:394-456.
2. Iglehart JK. Health policy report. New Jersey's experiment with DRG-based hospital reimbursement. N Engl J Med 1982;307:1655-60.
3. Grimaldi PL, Micheletti JA. DRG's diagnosis related groups: a practitioner's guide. Chicago: Pluribus Press, 1983:1-217.
4. DRGs and the prospective payment system: a guide for physicians. Chicago: American Medical Association, 1983:1-70.
5. Iglehart JK: The new era of prospective payment for hospitals. N Engl J Med 1982;307:1288-92.
6. Lagoe RJ: Obstetric hospital stays by diagnosis-related groups: a community-based analysis. Am J Obstet Gynecol 1986;154:873-8.
7. Goldberg HH. Prospective payment DRGs: New Jersey and the USA. Neurology 1984;34:1073-6.
8. Grimaldi PL, Micheletti JA. Homogeneity revisited. J Am Med Res Assoc 1982;53:56-70.
9. Grimaldi PL, Micheletti JA. On the homogeneity of DRGs. J Am Med Res Assoc 1980;51:43-9.
10. Grimaldi PL. Cluster approach allows budgeting, planning with DRGs. Hosp Prog 1984;65:74-9.
11. Health Care Consumer Protection Act of 1984, 2, Fl Stat Tit 395, §502.5.
12. Bauer G. The State of Florida's Regional Perinatal Tertiary Care Program: an overview. J Fla Med Assoc 1983;70:703-9.
13. Resnick MB, Ariet M, Carter RL, et al. Prospective pricing system for tertiary neonatal intensive care. Pediatrics 1986;78:820-8.
14. Resnick MB, Eitzman D, Dickman H, Ariet M, Ausbon WW. Data base management system for Children's Medical Services Regional Perinatal Intensive Care Centers Program. J Fla Med Assoc 1983;70:703-9.
15. PPS/DRG scoreboard for insurers. Hospitals 1984;58:52.
16. Matlin NM. Non-Medicare insurers moving to DRGs. Am Acad Pediatr Child Health Financing Rep 1985;2:2.

Fetal diaphragmatic hernia: Ultrasound diagnosis and clinical outcome in 19 cases

Beryl R. Benacerraf, M.D., and N. Scott Adzick, M.D.
Boston, Massachusetts

Nineteen cases of congenital diaphragmatic hernia diagnosed in utero are reported with emphasis on sonographic findings, associated congenital and karyotypic abnormalities, the presence or absence of polyhydramnios, and clinical outcome. The survival of these infants was very poor despite accurate prenatal diagnosis, maximal surgical and medical treatment and maximal postnatal care. The overall survival rate was 10.5%, and for fetuses who lived beyond delivery the survival rate was 20%. (Am J Obstet Gynecol 1987;156:573-6.)

Key words: Congenital defects, diaphragmatic hernia, ultrasound

Congenital diaphragmatic hernia is an anatomically simple defect that is easily correctable after birth by removal of the herniated viscera from the chest and repair of the hole in the diaphragm. However, most infants with congenital diaphragmatic hernia die of pulmonary insufficiency, despite immediate operation, because the lungs are too hypoplastic to support extrauterine life.[1, 2] Experimental studies have clearly shown that the pulmonary hypoplasia is a developmental consequence of fetal lung compression by the herniated viscera.[3] Prenatal detection and serial sonographic study of fetuses with congenital diaphragmatic

From Diagnostic Ultrasound Associates and the Department of Obstetrics and Gynecology, Brigham and Women's Hospital, and the Department of Surgery, Children's Hospital and Harvard Medical School.
Received for publication September 10, 1986; accepted October 6, 1986.
Reprint requests: Beryl R. Benacerraf, M.D., Diagnostic Ultrasound Associates, 333 Longwood Ave., Boston, MA 02115.

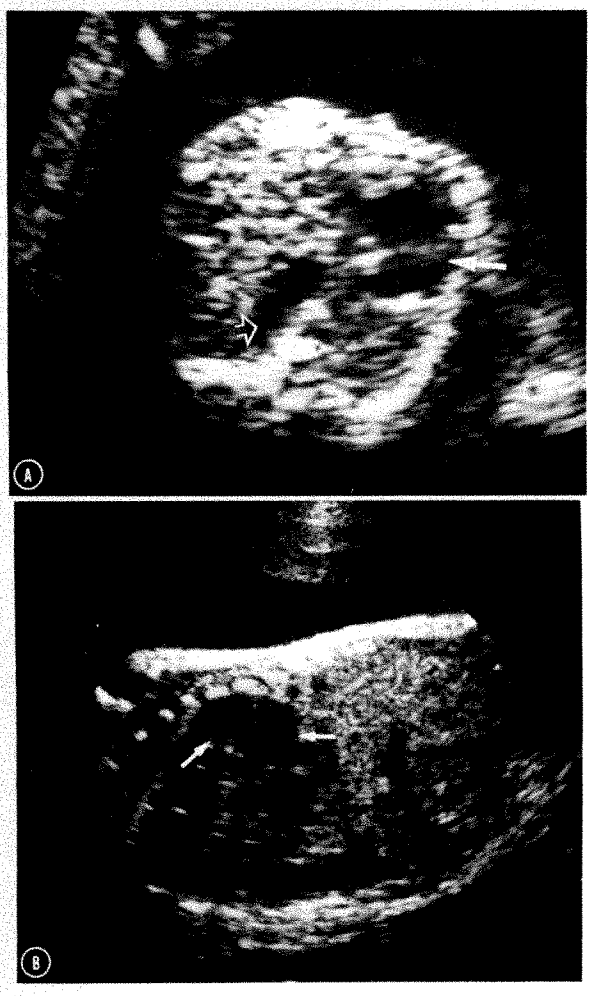


Fig. 1. *A*, Transverse view through the fetal chest at 18 weeks showing the heart deviated toward the right side *(solid arrow)* and the fetal stomach in the left side of the chest as shown by the *open arrow*. Incidental note is made that the heart had only two chambers, a double-outlet right ventricle with aortic atresia. *B*, Longitudinal view of a 32-week fetus showing the heart deviated against the chest wall *(arrows)*, with a large number of echo-poor bowel loops adjacent to the heart in the chest. The liver is noted in the abdomen below the heart.

hernia now make it possible to define the natural history of this lesion and determine the pathophysiologic features that affect clinical outcome.

We report 19 cases of congenital diaphragmatic hernia diagnosed in utero and describe the sonographic findings, associated congenital and karyotypic abnormalities, presence or absence of polyhydramnios, and clinical outcome. Our cases were diagnosed by a single ultrasonographer with postnatal treatment by the same surgical team. The survival of these infants was extremely poor despite early sonographic diagnosis, maternal transport, planned delivery, immediate resuscitation and operation, and maximal postnatal care, including the use of a newly developed extracorporeal membrane oxygenator.

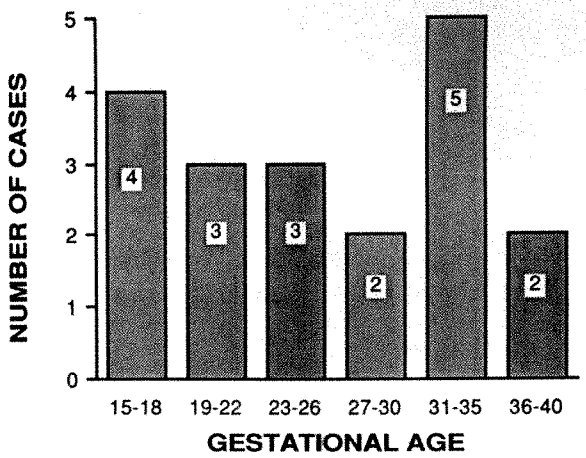


Fig. 2. Histogram showing the distribution of our 19 patients by gestational age in weeks.

Table I. Abnormal karyotypes

Karyotype	*No. of cases*	*Weeks' gestation at diagnosis*
Trisomy 18	2	15, 22
Trisomy 21	1	30
Tetraploidy 21	1	16

Material and methods

Over the course of 3½ years, 19 consecutive cases of congenital diaphragmatic hernia were diagnosed in a laboratory dedicated to high-risk obstetric sonography where more than 20,000 fetuses were scanned. The scans were performed with an Acuson 128, a variable focus phased-array system, and an ATL 300 IC, with 3.5 and 3 MHz transducers, respectively. The diagnosis of congenital diaphragmatic hernia was made when observation was made of an intrathoracic mass having the appearance of liver, bowel, or stomach, with displacement of the heart and mediastinum to the opposite side and with a paucity of abdominal contents in the abdomen (Fig. 1). Other features that were evaluated included amniotic fluid volume, evidence of other congenital malformations, karyotyping when amniocentesis was performed, and clinical outcome. All deliveries were performed at Brigham and Women's Hospital with planned neonatal resuscitation in the delivery room and immediate repair of congenital diaphragmatic hernia at the adjacent Children's Hospital.

Results

The sonographic diagnosis of congenital diaphragmatic hernia was made from 15 to 41 weeks' gestation, with seven being diagnosed between 15 and 21 weeks (Fig. 2). There were 14 fetuses with a left diaphragmatic hernia and five with a right diaphragmatic hernia.

Amniocentesis yielded four abnormal karyotypes:

Table II. Presence of polyhydramnios

Amount of fluid	*Before 24 wk*	*After 24 wk*
Polyhydramnios	1*	6
Normal fluid volume	4	5

Two cases did not have polyhydramnios in second trimester until 28 weeks. One case did not have fluid volume recorded.
*With anencephaly.

two cases of trisomy 18, diagnosed at 15 and 21 weeks; one case of tetraploidy 21, diagnosed at 16 weeks; and one case of trisomy 21, diagnosed at 30 weeks (Table I). Other congenital anomalies that were noted in nine fetuses included cardiac abnormalities, neural tube defects, omphalocele, hydrops, and multiple defects. There were 10 fetuses who had congenital diaphragmatic hernia as the only sonographic diagnosis (Fig. 3). The sonographic findings were confirmed by autopsy or clinical examination in all of these cases except for one fetus with trisomy 18 by karyotype that was too fragmented for pathologic examination after pregnancy termination.

Of the seven fetuses diagnosed with congenital diaphragmatic hernia before 22 weeks' gestation, none survived. Three had abnormal karyotypes (two with trisomy 18 and one with tetraploidy 21), and one fetus had anencephaly with a large encephalocele. All four of these fetuses were aborted. The remaining three fetuses in this group were carried to term. One fetus had severe spinal defects and was stillborn, and the other two had no other congenital abnormalities visualized on sonogram; one did not survive to leave the delivery room, and the other fetus died shortly after operation.

Polyhydramnios was present in only one fetus diagnosed before 24 weeks' gestation; this fetus had anencephaly, which probably caused the polyhydramnios (Table II). Two fetuses with the diagnosis made before 24 weeks (with no other congenital abnormalities) subsequently developed polyhydramnios between 28 and 30 weeks. Of the fetuses older than 24 weeks at diagnosis, the majority had polyhydramnios (six fetuses), and actually a total of eight had third-trimester polyhydramnios when the two cases that were diagnosed earlier are included. There were five fetuses diagnosed with congenital diaphragmatic hernia after 24 weeks that had normal amniotic fluid volumes.

Despite sophisticated perinatal management, the overall outcome was dismal (Table III). There were three cases of intrauterine fetal death or stillbirth. Two neonates deteriorated so rapidly after birth that surgical repair of the congenital diaphragmatic hernia could not be attempted. A total of eight infants died after operation, and severe pulmonary hypoplasia was

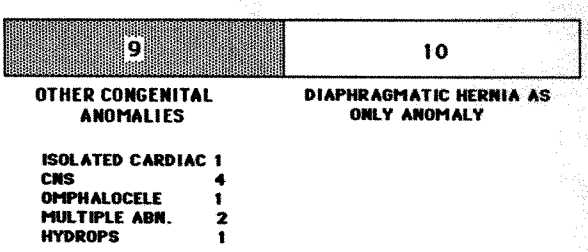


Fig. 3. Presence or absence of associated congenital abnormalities.

Table III. Outcomes

Outcome	*No. of cases*
Induced abortion	4
Intrauterine death	3
Died before operation	2
Died after operation	8*
Survived	2

*Two died before extracorporeal membrane oxygenation could be instituted; four died after a period of extracorporeal membrane oxygenation; two died despite maximal conventional treatment. The longest survival was 5 months.

confirmed at autopsy in each instance. The longest survival was 5 months with chronic respiratory insufficiency as the cause of death. Four of these neonates that underwent operation had the benefit of extracorporeal membrane oxygenation, and two others died before extracorporeal membrane oxygenation could be instituted.

Only two fetuses survived operation, and neither required extracorporeal membrane oxygenation for respiratory support (Table IV). Both cases were diagnosed prenatally during the late third trimester, and one had mild polyhydramnios.

Comment

The prenatal diagnosis of congenital diaphragmatic hernia was made in all 19 cases by identification of herniated abdominal viscera in the fetal chest, abnormal upper abdominal anatomy, and mediastinal shift away from the side of herniation. The diagnosis was made as early as 15 weeks' gestation, with polyhydramnios a common finding after 24 weeks. Since two fetuses with the early gestational diagnosis of congenital diaphragmatic hernia subsequently developed polyhydramnios at the beginning of the third trimester; this suggests that polyhydramnios is a relatively late manifestation of this disease. Consequently, careful examination of the fetal chest is necessary for congenital diaphragmatic hernia to be detected successfully and reliably in the second trimester. Previous work has demonstrated that polyhydramnios not only is a marker for fetal congenital diaphragmatic hernia but also is a good predictor for severe cases with poor prognosis.[4] Al-

Table IV. Survivors

Age at diagnosis	*Hernia side*	*Other abnormality*	*Polyhydramnios*	*Outcome*
37	Left	No	Yes (mild)	Did well, extracorporeal membrane oxygenation not needed
32	Left	No	No	Did well, extracorporeal membrane oxygenation not needed

though the cause of polyhydramnios is unclear in congenital diaphragmatic hernia, functional upper gastrointestinal obstruction from the bowel herniation probably plays a role.

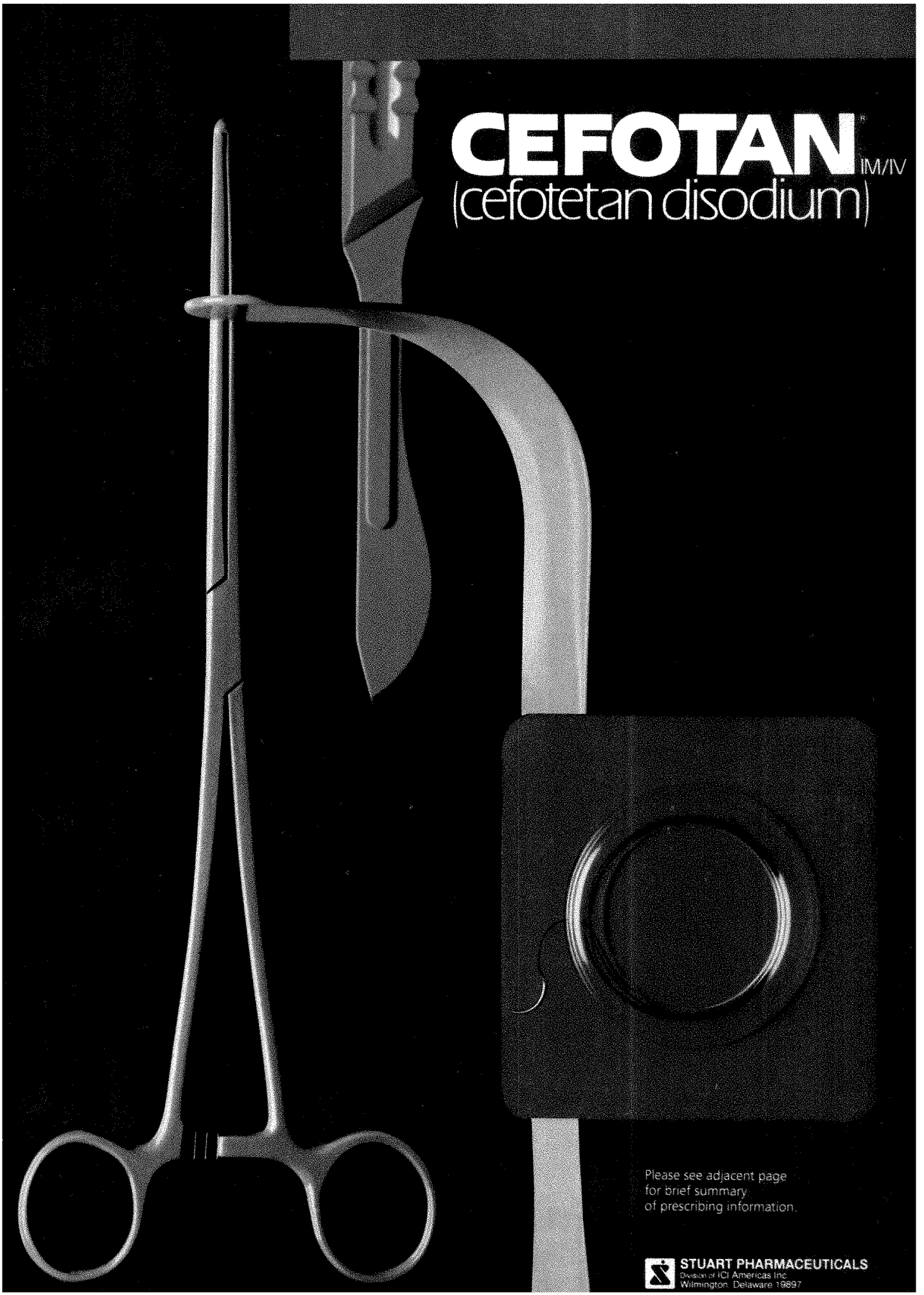
Previous reports have noted that the incidence of associated anomalies with congenital diaphragmatic hernia have varied from "rare" to 56%.[5-7] Current prenatal diagnostic techniques can detect these structural defects, and all were accurately diagnosed in our series. It is noteworthy that 4/19 or 21% of these patients had chromosomal abnormalities, suggesting that amniocentesis for karyotyping is indicated when congenital diaphragmatic hernia is diagnosed antenatally.

The prenatal diagnosis of congenital diaphragmatic hernia has been reported in several case studies and small series.[8-11] The largest series is a multicenter survey of 94 cases, in which 90% of the cases had optimal perinatal care with maternal transport, planned delivery, and immediate resuscitation.[4] Despite sophisticated postnatal therapy, only 20% of these babies survived. Our study describes a series of patients in which the diagnosis of congenital diaphragmatic hernia was made by a single ultrasonographer and where each fetus coming to delivery had maximal resuscitation and immediate operation by the same surgical team. In addition, the membrane oxygenator was available for 1 to 2 weeks of complete postoperative respiratory support for neonates with failure of conventional ventilatory management. Despite these considerations, the overall survival rate was 10.5% (2/19); for fetuses that lived beyond delivery, the survival rate was 20% (2/10).

In the future, prenatal diagnosis may permit surgical intervention before birth. A crucial part of fetal lung development occurs in the middle of the second trimester.[12] If the diagnosis of congenital diaphragmatic hernia is made early, it may be possible to intervene in the second trimester rather than waiting until birth where, despite maximal treatment, survival has not improved. Harrison et al.[13] and Adzick et al.[14] have described the surgical reduction of congenital diaphragmatic hernia in utero in a fetal lamb model, permitting lung development to take place during the remainder of pregnancy with survival at birth. In addition, they have demonstrated the safety of hysterotomy and the control of preterm labor in the nonhuman primate.[15, 16] If this approach is contemplated, early and accurate diagnosis of fetal congenital diaphragmatic hernia is essential even before the onset of polyhydramnios. This approach would require amniocentesis to exclude chromosomal abnormalities as well as careful fetal sonography to detect other structural abnormalities. For the present, the poor prognosis of fetal congenital diaphragmatic hernia requires objective counseling of the parents, particularly when options for pregnancy termination are available.

REFERENCES

1. Harrison MR, deLorimier AA. Congenital diaphragmatic hernia. Surg Clin North Am 1981;61:1023-35.
2. Harrison MR, Bjordal RI, Landmark F, et al. Congenital diaphragmatic hernia: the hidden mortality. J Pediatr Surg 1979;13:227-30.
3. Harrison MR, Jester JA, Ross NA. Correction of congenital diaphragmatic hernia in utero. I. The model: intrathoracic balloon produces fetal pulmonary hypoplasia. Surgery 1980;88:174-82.
4. Adzick NS, Harrison MR, Glick PL, et al. Diaphragmatic hernia in the fetus: prenatal diagnosis and outcome in 94 cases. J Pediatr Surg 1985;20:357-61.
5. Fitzgerald RJ. Congenital diaphragmatic hernia: a study of mortality factors. Isr J Med Sci 1979;146:280-98.
6. Butler N, Claireaux AE. Congenital diaphragmatic hernia as a cause of perinatal mortality. Lancet 1962;1:659-71.
7. Puri P, Gorman F. Lethal nonpulmonary anomalies associated with congenital diaphragmatic hernia: implications for early intrauterine surgery. J Pediatr Surg 1984;19:29-46.
8. Chinn DH, Filly RA, Callen PW, et al. Congenital diaphragmatic hernia diagnosed prenatally by ultrasound. Radiology 1983;148:199-203.
9. Marwood RP, Davison OW. Antenatal diagnosis of diaphragmatic hernia: case report. Br J Obstet Gynaecol 1981;88:77-82.
10. Benacerraf BR, Greene MF. Congenital diaphragmatic hernia: US diagnosis prior to 22 weeks gestation. Radiology 1986;158:809-10.
11. Stiller RJ, Roberts NS, Weiner S, et al. Congenital diaphragmatic hernia: antenatal diagnosis and obstetrical management. JCU 1985;13:212-5.
12. Inselman LS, Mellins RB. Growth and development of the lung. J Pediatr 1981;98:1-17.
13. Harrison MR, Adzick NS, Nakayama DK. Fetal diaphragmatic hernia: fatal but fixable. Semin Perinatol 1985;9:103-12.
14. Adzick NS, Outwater K, Harrison MR, et al. Correction of congenital diaphragmatic hernia in utero. IV. An early gestational model for pulmonary vascular morphometric analysis. J Pediatr Surg 1985;20:673-80.
15. Harrison MR, Anderson J, Rosen MA, et al. Fetal surgery in the primate. I. Anesthetic, surgical, and tocolytic management to maximize fetal-neonatal survival. J Pediatr Surg 1982;17:115-22.
16. Adzick NS, Harrison MR, Glick PL, et al. Fetal surgery in the primate. III. Maternal outcome after fetal surgery. J Pediatr Surg 1986;21:477-80.


CEFOTAN®
IM/IV
(cefotetan disodium)
Please see adjacent page
for brief summary
of prescribing information.
STUART PHARMACEUTICALS
Division of ICI Americas Inc.
Wilmington, Delaware 19897

In intra-abdominal and gynecologic infection due to indicated organisms

Switch to

CEFOTAN® IM/IV
(cefotetan disodium)

The cost-effective replacement for cefoxitin

For Intravenous or Intramuscular Use

BRIEF SUMMARY

INDICATIONS AND USAGE
TREATMENT: CEFOTAN® (cefotetan disodium) is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:

Urinary tract infections caused by *E coli*, *Klebsiella* species (including *K pneumoniae*), *Proteus mirabilis* and *Proteus* sp (which may include the organisms now called *Proteus vulgaris*, *Providencia rettgeri*, and *Morganella morganii*).

Lower respiratory tract infections caused by *Streptococcus pneumoniae* (formerly *D pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K pneumoniae*), and *E coli*.

Skin and skin structure infections caused by *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis*,* *Streptococcus pyogenes* and *Streptococcus* species (excluding enterococci), and *E coli*.

Gynecologic infections caused by *Staphylococcus aureus*† (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis*,* *Streptococcus* species (excluding enterococci), *E coli*, *Proteus mirabilis*, *Neisseria gonorrhoeae*, *Bacteroides* species (excluding *B distasonis*, *B ovatus*, *B thetaiotaomicron*), *Fusobacterium* species,† and gram-positive anaerobic cocci (including *Peptococcus* and *Peptostreptococcus* species†).

Intra-abdominal infections caused by *E coli*, *Klebsiella* species (including *K pneumoniae*†), *Streptococcus* species (excluding enterococci), and *Bacteroides* species (excluding *B distasonis*, *B ovatus*, *B thetaiotaomicron*).

Bone and joint infections caused by *Staphylococcus aureus* †

***NOTE:** Some strains of *Staphylococcus epidermidis* are resistant to cefotetan.

†Efficacy for this organism in this organ system was studied in fewer than ten infections.

PROPHYLAXIS: The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (eg, cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS
CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS
Before therapy with CEFOTAN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefotetan disodium, cephalosporins, penicillins, or other drugs. This product should be given cautiously to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to CEFOTAN occurs, discontinue the drug. Serious acute hypersensitivity reactions may require epinephrine and other emergency measures.

Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with antibiotic use.

Treatment with broad-spectrum antibiotics may alter normal flora of the colon and may permit overgrowth of clostridia. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin in vitro.

Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte, and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance, or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C difficile*. Other causes should also be considered.

PRECAUTIONS
GENERAL: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity. Those at risk include patients with renal or hepatic impairment, or poor nutritional state. Prothrombin times should be monitored in patients at risk and exogenous Vitamin K administered as indicated.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

INFORMATION FOR PATIENTS: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.

DRUG INTERACTIONS: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycoside antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

DRUG/LABORATORY TEST INTERACTIONS: A false positive reaction for glucose in urine may occur with Benedict's or Fehling's solution.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels of Jaffe reaction and produce false increases in the levels of creatinine reported.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approx 8-16 times the usual adult human dose) on days 6-35 of life (thought to be developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approx 2-4 times the usual human dose), only 1 of 10 treated animals was affected, and the degree of degeneration was mild. Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.

USAGE IN PREGNANCY: Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

USAGE IN NURSING MOTHERS: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.

PEDIATRIC USE: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS
In clinical studies the following adverse effects were considered related to CEFOTAN therapy.

Gastrointestinal symptoms occurred in 1.5 percent of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700).

Hematologic laboratory abnormalities occurred in 1.4 percent of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300).

Hepatic enzyme elevations occurred in 1.2 percent of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).

Hypersensitivity reactions were reported in 1.2 percent of patients and included rash (1 in 150) and itching (1 in 700).

Local effects were reported in less than one percent of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500).

DOSAGE AND ADMINISTRATION
TREATMENT: The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6* grams	3 g every 12 hours IV

*Maximum daily dosage should not exceed 6 grams.

PROPHYLAXIS: To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.

IMPAIRED RENAL FUNCTION: When renal function is impaired, a reduced dosage schedule must be employed. The following dosage guidelines may be used.

DOSAGE GUIDELINES FOR PATIENTS WITH IMPAIRED RENAL FUNCTION

Creatinine Clearance mL/min	Dose	Frequency
>30	Usual Recommended Dose*	Every 12 hours
10-30	Usual Recommended Dose*	Every 24 hours
<10	Usual Recommended Dose*	Every 48 hours

*Dose determined by the type and severity of infection, and susceptibility of the causative organism.

Cefotetan is dialyzable and it is recommended that for patients undergoing intermittent hemodialysis, one-quarter of the usual recommended dose be given every 24 hours on days between dialysis and one-half the usual recommended dose on the day of dialysis.

HOW SUPPLIED
CEFOTAN is a dry, white to pale yellow powder supplied in vials containing cefotetan disodium equivalent to 1 g and 2 g cefotetan activity for intravenous and intramuscular administration. The 1 g dose is available in 10 mL and 100 mL vials, and the 2 g dose is available in 20 mL and 100 mL vials. The vials should not be stored at temperatures above 22°C and should be protected from light.
1 g in 10 mL vial (NDC 0038-0376-10)
2 g in 20 mL vial (NDC 0038-0377-20)
1 g in 100 mL vial (NDC 0038-0376-11)
2 g in 100 mL vial (NDC 0038-0377-21)

Manufactured for:

STUART PHARMACEUTICALS
Division of ICI Americas Inc
Wilmington, Delaware 19897

Rev. E 3/86 63967-02

CEFOTAN® (cefotetan disodium)

HUMAN MILK IN INFANT NUTRITION AND HEALTH *edited by* **R. Rodney Howell, Frank H. Morriss, Jr.,** *and* **Larry K. Pickering.** The contributors report on investigations into the characteristics of human milk that exert specific beneficial effects on newborn infants. They describe the mechanisms that produce such effects, and they direct special attention to women who delivered before term. *'86, $49.00*

COLPOSCOPY: A Scientific and Practical Approach to the Cervix, Vagina and Vulva in Health and Disease (3rd Ed.) *by* **Malcolm Coppleson, Ellis Pixley** *and* **Bevan Reid.** Precise histological correlations, excellent atlas features, and a sound approach to detection and management make this book essential reading to all who practice colposcopy. *'86, $71.25*

VAGINAL HYSTERECTOMY: Indications, Technique, and Complications (3rd Ed.) *by* **Laman A. Gray.** This thorough Third Edition encompasses indications for vaginal hysterectomy; discusses the use of adrenalin to conserve blood; recommends prophylactic antibodies; and details complications related to cystocele and rectocele repair. *'83, $53.75*

We fill all orders promptly • Books sent on approval
MasterCard, Visa & prepaid orders sent postpaid
Catalog sent on request • Write or call (217) 789-8980

CHARLES C THOMAS • PUBLISHER
2600 South First Street
Springfield • Illinois • 62794-9265

Good News For Ob/Gyns

Informed Consent™ Systems Videotapes:

- ✓ reflect current patient education trends
- ✓ save time for the doctor and staff
- ✓ offer improved legal protection for the doctor
- ✓ enhance goodwill with patients
- ✓ use easy-to-understand diagrams and graphics
- ✓ relieve patient worry and stress
- ✓ improve comprehension thru visual presentation
- ✓ include complimentary documentation forms

Interested?
Call 1-800-FILMTEC
$25 off first purchase

INFORMED CONSENT™ SYSTEMS

Distributed by FILMTEC, INC.
11250-22 Roger Bacon Drive
Reston, Virginia 22090
(703) 471-6890

POSITION AVAILABLE

A full-time position in the area of Reproductive Biology/Endocrinology is available in the Department of OB/GYN, U.C. Davis Medical Center, Sacramento, California. Applicants should be board eligible or certified in Obstetrics and Gynecology and preferably completed subspecialty training in Reproductive Endocrinology. Applicants must demonstrate skill and competence in microsurgery and be knowledgeable in the area of in vitro fertilization.

Responsibilities include patient case management, participation in teaching activities, development and/or participation in research activities and involvement in University and Hospital committees.

Send curriculum vitae and five references to:

Edward J. Quilligan, M.D.
Chairman, Department of Obstetrics and Gynecology
University of California, Davis, Medical Center
1621 Alhambra Boulevard, Suite 2500
Sacramento, California 95816

The final date for receipt of applications is "Open until filled, but not later than May 1, 1987."

The University of California is an Affirmative Action/ Equal Opportunity Employer.

Position:	Professor and Head Department of Obstetrics and Gynecology
Responsibilities To Be Fulfilled:	Direct the education, research and service programs of an Obstetrics and Gynecology Department in the College of Medicine.
Qualifications Desired:	M.D. degree, board certification or equivalent and demonstrated clinician, scholar and leader in Obstetrics and Gynecology.
Salary:	Appropriate for qualifications and responsibilities for a Professor and Head of Obstetrics and Gynecology. Excellent fringe benefits.
Employer:	The University of Iowa, Iowa City, Iowa 52242 is an Affirmative Action/ Equal Opportunity Employer. Women and minorities are encouraged to apply.
Application:	Interested persons are invited to direct inquiries and submit vitae to: Dr. John Tinker, Professor and Head Department of Anesthesia College of Medicine SE618 University Hospital The University of Iowa Iowa City, Iowa 52242

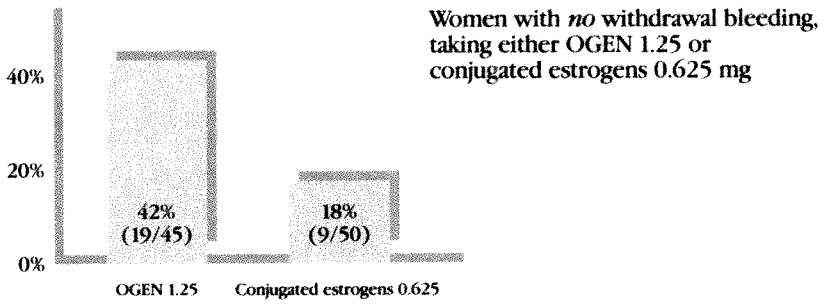
Withdrawal Bleeding in the ERT Patient:

Necessity or Nuisance?

▣ **To protect against the endometrial hyperplasia** that may result from continuous, unopposed estrogen administration, cyclical estrogen/progestogen therapy is often given.[1] But this may induce withdrawal bleeding, unacceptable to many patients.

▣ **Progestogen initiates bleeding** only when the endometrial mucosa is stimulated to the threshold level by the estrogen.

▣ **Less withdrawal bleeding was experienced** by patients given estrone plus a progestogen than by those given conjugated estrogens plus a progestogen.[2,3] Studies suggest that at doses that control symptoms, estrone alone is less uterotrophic than conjugated estrogens.



Patients received either OGEN or conjugated estrogens 24 days out of 30 for three months. A progestogen, levonorgestrel 0.03 mg, was added from the 15th to the 24th days. Adapted from Wren et al.[2]

▣ **OGEN® (estropipate) may produce less withdrawal bleeding** *at doses that effectively protect them from the symptoms of menopause.*

7013807

Abbott Pharmaceuticals, Inc.
North Chicago, Illinois 60064



OGEN® (estropipate) Induces Less Withdrawal Bleeding

OGEN®
(estropipate)

Only estrone. Identical to the body's own.

OGEN®

ESTROPIPATE TABLETS, USP

Tablets

WARNING:

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. OGEN SHOULD NOT BE USED DURING PREGNANCY.

According to some investigators, the use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. Studies have reported that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. In one of these studies, this risk was estimated as not greater than 4 per 1000 exposures. Furthermore, there are reports that a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these reported changes are histologically benign, the investigators have not determined whether they are precursors of adenocarcinoma.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies in the offspring, including heart defects and limb reduction defects. One case control study estimated a 4.7 fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. OGEN has not been studied for these uses, and therefore should not be used during pregnancy. There is no evidence from well controlled studies that progestogens are effective for these uses.

If OGEN (estropipate tablets) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the question of continuation of the pregnancy should be addressed.

INDICATIONS AND USAGE

The cyclic administration of OGEN (estropipate tablets) is indicated for the treatment of estrogen deficiency associated with (See "DOSAGE AND ADMINISTRATION" section):

1. Moderate to severe *vasomotor* symptoms of menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.)
2. Atrophic vaginitis.
3. Kraurosis vulvae.
4. Female hypogonadism.
5. Female castration.
6. Primary ovarian failure.

OGEN (ESTROPIPATE TABLETS) HAS NOT BEEN TESTED FOR EFFICACY FOR ANY PURPOSE DURING PREGNANCY. SINCE ITS EFFECT UPON THE FETUS IS UNKNOWN, IT CANNOT BE RECOMMENDED FOR ANY CONDITION DURING PREGNANCY (SEE BOXED WARNING).

CONTRAINDICATIONS

OGEN should not be used in women with any of the following conditions:

1. Known or suspected cancer of the breast.
2. Known or suspected estrogen-dependent neoplasia.
3. OGEN may cause fetal harm when administered to a pregnant woman. OGEN is contraindicated in women who are or may become pregnant (See Boxed Warning).
4. Undiagnosed abnormal genital bleeding.
5. Active thrombophlebitis or thromboembolic disorders.
6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use.

WARNINGS

1. *Induction of malignant neoplasms.* Long-term continuous administration of natural and synthetic estrogens in certain animal species has been reported by some investigators to increase the frequency of carcinomas of the breast, cervix, vagina, and liver. There is now evidence that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning).

At the present time there is no conclusive evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast. There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer among these women. Therefore, caution should be exercised when administering estrogens to women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. Careful breast examinations should be performed periodically.

2. *Gall bladder disease.* A recent study has reported a 2 to 3-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens, similar to the 2-fold increase previously noted in users of oral contraceptives. In the case of oral contraceptives, the increased risk appeared after two years of use.

3. *Effects similar to those caused by estrogen-progestogen oral contraceptives.* There are several serious adverse effects of oral contraceptives, most of which have not, up to now, been documented as consequences of postmenopausal estrogen therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women. It would be expected that the larger doses of estrogen used to treat postpartum breast engorgement would be more likely to result in these adverse effects, and, in fact, it has been shown that there is an increased risk of thrombosis in women receiving estrogens for postpartum breast engorgement.

a. *Thromboembolic disease.* It is now well established that users of oral contraceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism; it should also be discontinued during periods of prolonged immobilization.

While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found this does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be used in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men to increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

b. *Hepatic adenoma.* Benign hepatic adenomas appear to be associated with the use of oral contraceptives. Although benign, and rare, these may rupture and cause death through intraabdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations but should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has also been reported in women taking estrogen containing oral contraceptives. The relationship of this malignancy to these drugs is not known at this time.

c. *Elevated blood pressure.* Increased blood pressure is not uncommon in women using oral contraceptives. There is now a report that this may occur with use of estrogens in the menopause and blood pressure should be monitored with estrogen use, especially if high doses are used.

d. *Glucose tolerance.* A worsening of glucose tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen.

4. *Hypercalcemia.* Administration of estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases. If this occurs, the drug should be stopped and appropriate measures taken to reduce the serum calcium level.

PRECAUTIONS

A. General Precautions.

1. A complete medical and family history should be taken prior to the initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolau smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed.

2. Fluid retention — Estrogens may cause some degree of fluid retention. Therefore, patients with conditions such as epilepsy, migraine, and cardiac or renal dysfunction, which might be influenced by this factor, require careful observation.

3. Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc.

4. Oral contraceptives appear to be associated with an increased incidence of mental depression. Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.

5. Preexisting uterine leiomyomata may increase in size during estrogen use.

6. The pathologist should be advised of the patient's use of estrogen therapy when relevant specimens are submitted.

7. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving estrogen-containing oral contraceptive therapy. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated.

8. Estrogens may be poorly metabolized in patients with impaired liver function and they should be administered with caution in such patients.

9. Because estrogens influence the metabolism of calcium and phosphorus, they should be used with caution in patients with metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency.

B. Information for the Patient. See text of Patient Package Insert which appears after PHYSICIAN REFERENCES.

C. Drug Interactions. The concomitant use of any drugs which can induce hepatic microsomal enzymes with estrogens may produce estrogen levels which are lower than would be expected from the dose of estrogen administered.

The use of *broad spectrum antibiotics* which profoundly effect intestinal flora may influence the absorption of steroidal compounds including the estrogens.

Diabetics receiving *insulin* may have increased insulin requirements when receiving estrogens.

Laboratory Test Interference. Certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The following similar changes may be expected with larger doses of estrogen:

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.

d. Abnormal glucose tolerance test results.

e. Decreased pregnanediol excretion.

f. Reduced response to metyrapone test.

g. Reduced serum folate concentration.

h. Increased serum triglyceride and phospholipid concentration.

D. Carcinogenesis. Studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods (see Boxed Warning). At the present time there is no conclusive evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast. There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer among these women. (See "WARNINGS" section.)

E. Pregnancy. Pregnancy Category X. See "CONTRAINDICATIONS" section and Boxed Warning.

F. Nursing Mothers. Estrogens have been reported to be excreted in human breast milk. Caution should be exercised when OGEN is administered to a nursing woman.

G. Pediatric Use. Because of the effects of estrogens on epiphyseal closure, they should be used judiciously in young patients in whom bone growth is not complete.

ADVERSE REACTIONS

(See Warnings regarding reports of possible induction of neoplasia, unknown effects upon the fetus, increased incidence of gall bladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.) The following additional adverse reactions in decreasing order of severity within each category have been reported with estrogenic therapy, including oral contraceptives:

1. *Genitourinary system.*
Increase in size of uterine fibromyomata.
Vaginal candidiasis.
Cystitis-like syndrome.
Dysmenorrhea.
Amenorrhea during and after treatment.
Change in cervical eversion and in degree of cervical secretion.
Breakthrough bleeding, spotting, change in menstrual flow.
Premenstrual-like syndrome.
2. *Breast.*
Tenderness, enlargement, secretion.
3. *Gastrointestinal.*
Cholestatic jaundice.
Vomiting, nausea.
Abdominal cramps, bloating.
4. *Skin.*
Hemorrhagic eruption.
Erythema nodosum.
Erythema multiforme.
Hirsutism.
Chloasma or melasma which may persist when drug is discontinued.
Loss of scalp hair.
5. *Eyes.*
Steepening of corneal curvature.
Intolerance to contact lenses.
6. *CNS.*
Chorea.
Mental depression.
Migraine, dizziness, headache.
7. *Miscellaneous.*
Aggravation of porphyria.
Edema.
Reduced carbohydrate tolerance.
Increase or decrease in weight.
Changes in libido.

OVERDOSAGE

Numerous reports of ingestion of large doses of estrogen-containing oral contraceptives by young children indicate that serious ill effects do not occur. Overdosage of estrogen may cause nausea and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION

1. *Given cyclically for short-term use:*

For treatment of moderate to severe *vasomotor* symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (e.g., 3 weeks on and 1 week off).

Attempts to discontinue or taper medication should be made at 3 to 6 month intervals.

Usual dosage ranges:

Vasomotor symptoms —One OGEN .625 (estropipate) Tablet to one OGEN 5 Tablet per day. The lowest dose that will control symptoms should be chosen. If the patient has not menstruated within the last two months or more, cyclic administration is started arbitrarily. If the patient is menstruating, cyclic administration is started on day 5 of bleeding.

Atrophic vaginitis and kraurosis vulvae — One OGEN .625 Tablet to one OGEN 5 Tablet daily, depending upon the tissue response of the individual patient. The lowest dose that will control symptoms should be chosen. Administer cyclically.

2. *Given cyclically:*

Female hypogonadism; female castration; primary ovarian failure.

Usual dosage ranges:

Female hypogonadism — A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. The lowest dose that will control symptoms should be chosen. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the responsiveness of the endometrium. If satisfactory withdrawal bleeding does not occur, an oral progestogen may be given in addition to estrogen during the third week of the cycle.

Female castration and primary ovarian failure —A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. Adjust dosage upward or downward according to severity of symptoms and response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

HOW SUPPLIED

OGEN (estropipate tablets, USP) is supplied as OGEN .625 (0.75 mg estropipate), yellow tablets, **NDC** 0074-3943-04; OGEN 1.25 (1.5 mg estropipate), peach-colored tablets, **NDC** 0074-3946-04; OGEN 2.5 (3 mg estropipate), blue tablets, **NDC** 0074-3951-04; and OGEN 5 (6 mg estropipate), light green tablets, **NDC** 0074-3958-13. Tablets of all four dosage levels are standardized to provide uniform estrone activity and are grooved (Divide-Tab®) to provide dosage flexibility. All tablet sizes of OGEN are available in bottles of 100.

7013807

References:

1. Whitehead MI, Townsend PT, Pryse-Davies J, et al: Actions of progestins on the morphology and biochemistry of the endometrium of postmenopausal women receiving low-dose estrogen therapy. *Am J Obstet Gynecol* 142:791-795, 1982.

2. Wren BG, Brown LB, Routledge DA: Differential clinical response to oestrogens after menopause. *Med J Aust* 2:329-332, 1982.

3. Lind T, Cameron EC, Hunter WM, et al: A prospective, controlled trial of six forms of hormone replacement therapy given to postmenopausal women. *Br J Obstet Gynaecol* 86(suppl 3):1-29, 1979.

Abbott Laboratories
Abbott Pharmaceuticals, Inc.
North Chicago, Illinois 60064

A viable pregnancy in a patient with myositis ossificans progressiva

Yvonne S. Thornton, M.D., Stanley J. Birnbaum, M.D., and Nancy Lebowitz, M.D.
New York, New York

Myositis ossificans progressiva is a rare autosomal dominant disorder characterized by a progressive ectopic ossification of the connective tissue of the voluntary muscles and ligaments with associated debilitation and characteristic skeletal malformations. There have been less than 600 cases in the literature. This is the first reported case of a viable pregnancy in a patient with myositis ossificans progressiva. (Am J Obstet Gynecol 1987;156:577-8.)

Key words: Myositis ossificans progressiva and pregnancy, fibrodysplasia ossificans progressiva and pregnancy, pregnancy

Myositis ossificans progressiva, also known as fibrodysplasia ossificans progressiva, is usually manifested before the age of 20 in 98% of patients. The clinical features include microdactyly, reduction defects of all digits, deafness, baldness of the scalp, and mental retardation. The term myositis is misleading, since most of the pathologic features suggest primary involvement of the connective tissue and the muscles are not inflamed. The major defect is dysplasia of fibrous and connective tissue. The initial manifestation closely resembles that produced by inflammation of the muscles, being red, swollen, and painful. The progressive nature of this debilitating disease soon demonstrates fixation of one or both hips, usually during adolescence.

Life expectancy is thought to be shortened in the patient with myositis ossificans progressiva. Patients usually die of respiratory infections and pneumonia due to the general immobility and complete fixity of the chest. This is the first reported case of a viable pregnancy in a patient with myositis ossificans progressiva.

Case report

A. M., a 27-year-old white, Rh-negative woman, gravida 2, para 0-0-1-0, whose last menstrual period was October 1985, was transferred to our medical center at 26 weeks of gestation with a 10-day history of spontaneous rupture of the membranes. The patient had been diagnosed as having myositis ossificans progressiva since the age of 3 with ankylosis of the spine and temporomandibular joint as well as combined restrictive and obstructive pulmonary disease. The patient was fixed in the fetal position with marked rigidity and had been confined to a wheelchair since 1975, with minimal use of the left lower extremity and the distal upper extremities. The patient had absolute inability to abduct the hips. Conception occurred in the knee-chest position.

Her past obstetric history was significant for a spontaneous abortion at 16 weeks of gestation in 1983, which was uncomplicated and did not require dilatation and curettage.

This pregnancy was uncomplicated until 10 days before her transfer, when she had spontaneous rupture of the membranes. She presented to the referring hospital 2 days after rupture of the membranes. Cultures at that time were positive for group B β-hemolytic streptococci, for which she had received amoxicillin for 7 days.

After administration of dexamethasone, the patient was transferred to our obstetric service for further management of this complicated pregnancy.

Physical examination on admission revealed stable vital signs with a temperature of 36° C. The patient was wheelchair bound with multiple contractures and severe involvement of the elbows and lower extremities. The patient was unable to open the mouth or to flex or extend the neck. The lungs had decreased breath sounds at both bases. The fetal heart rate was 130 bpm. Anesthesia, rheumatology, pulmonary disease, and otorhinolaryngology consults were obtained. The patient had widespread multiple areas of heterotopic bone formation with ankylosis of the temporomandibular joint and entire spine, from the cervical to the sacral region, secondary to ossification. Ultrasound examination at the time of transfer to our hospital revealed a biparietal diameter consistent with 26.4 weeks' gestation. The estimated fetal weight was 770 gm with decreased amniotic fluid.

The major concern about this patient was the mode of delivery and the anesthesia required to effect the delivery. Vaginal delivery was impossible because of the total inability of the patient to abduct the hips. Regional

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, The New York Hospital–Cornell Medical Center.
Received for publication September 4, 1986; accepted September 19, 1986.
Reprint requests: Yvonne S. Thornton, M.D., The New York Hospital–Cornell Medical Center, 525 East 68th St., Room M0032, New York, NY 10021.

block was impossible because of the ankylosis of the entire spine. Oral intubation was not feasible because of the temporomandibular joint ankylosis. Fiberoptic guided nasal intubation with a bronchoscope was a possibility but there was a significant risk of bleeding and/or trauma with subsequent airway occlusion. Tracheostomy was not considered because of possible ossification of the trachea, which would jeopardize the airway in the future. Therefore local anesthesia was the only safe alternative for this patient.

During her hospitalization, the patient remained afebrile, and the leukocyte count remained within normal limits. Results of a C-reactive protein test were positive, and vaginal cultures were negative for group B β-hemolytic streptococci. The plan of management was conservative. The patient had serial antepartum fetal heart rate tests and ultrasonographic evaluation that revealed appropriate fetal growth with reactive nonstress testing. An elective cesarean section was to be performed when the patient completed 30 weeks of gestation; however, 1 week before that stage of gestation, the patient had a nonreactive nonstress test. A spontaneous contraction stress test was negative and the biophysical profile score was 6. With the possibility of imminent delivery, the patient was given Celestone and continuously monitored. During the continuous monitoring, the fetal heart rate showed several late decelerations and the decision was made to perform a cesarean section for fetal stress.

Local anesthetic was administered, with a dose of 5 to 6 mg/kg of 0.5% to 0.75% lidocaine (5 to 7.5 mg/ml) in order to avoid local anesthetic toxicity. A total of 50 to 60 ml of 0.5% lidocaine infiltrated the skin to the peritoneum and a classical cesarean section was performed with a delivery of a live female infant weighing 1165 gm. Apgar scores were 6 and 9, at 1 and 5 minutes, respectively. The cesarean section proceeded without incident and a bilateral tubal ligation was performed at that time.

The patient's course was benign with good wound healing. She received Rh immune globulin and was discharged in good condition without worsening of pulmonary status. The infant was taken to the neonatal intensive care unit for observation. The 29-week female infant had mild respiratory distress and abnormal posturing and alignment secondary to the decreased intrauterine amniotic fluid. The sepsis evaluation was negative. There were no skeletal abnormalities. The infant continued to thrive and was discharged after 30 days, weighing 1520 gm, to be followed up in the nursery of the referring hospital.

Comment

Myositis ossificans progressiva is an autosomal dominant disorder and it has been suggested that advanced paternal age is the most important factor in the occurrence of new mutations. Contrary to an earlier report of Connor and Evans,[1] patients with myositis ossificans progressiva are fertile and are capable of maintaining a pregnancy.

In this patient with myositis ossificans progressiva, as pregnancy progressed with uterine enlargement, worsening of restrictive pulmonary disease was inevitable. With the complete immobility of the patient, the pregnancy also placed her at a higher risk for thromboembolic disease. Smith[2] reported exacerbation of the myositis ossificans, as well as poor wound healing and ossification of the scar tissue after operation. This was not the case in this patient.

A major concern in this case was the risk of anesthesia. This was minimized by the use of local anesthetic with an emergency support system of fiberoptic nasal intubation.

The management of myositis ossificans progressiva is conservative. Surgical removal of ectopic bone alone is invariably followed by recurrent ossification and should therefore be avoided.

Despite the good outcome in this case, the potential life-threatening risks of pregnancy should be discussed with the patient with myositis ossificans progressiva before conception. Because this is an autosomal dominant disease that carries a 50% risk of transmission to offspring in addition to severe physical limitations that significantly impair the patient's ability to care for her infant, elective sterilization should also be discussed with the patient.

We would like to thank Dr. Hamid Mootabar for referring this most interesting patient to our obstetric service for definitive care and delivery.

REFERENCES

1. Connor JM, Evans DAP. Fibrodysplasia ossificans progressiva: the clinical features and natural history of 34 patients. J Bone Joint Surg 1982;64B:76-83.
2. Smith R. Myositis ossificans progressiva: a review of current problems. Semin Arthritis Rheum 1975;4:369-80.

Can ultrasonography replace amniocentesis in fetal gender determination during the early second trimester?

E. Albert Reece, M.D., Hung N. Winn, M.D., Macor Wan, M.D., Carol Burdine, R.D.M.S., Jacqueline Green, M.S., R.D.M.S., and John C. Hobbins, M.D.

New Haven, Connecticut

Fetal gender was prospectively determined by ultrasonography in 115 patients with singleton pregnancies between 16 and 20 weeks of gestation. Gender determination was performed as a part of the sonographic examination preceding genetic amniocentesis, and the results were compared to the amniotic fluid fetal karyotype results. Our rate of visualization of the fetal external genitalia was 83.5%. The accuracy rate for gender determination was 90% in male infants and 100% in female infants, with an overall rate of prediction of 92.7%. (Am J Obstet Gynecol 1987;156:579-81.)

Key words: Ultrasound, fetal sex, fetal gender, prenatal diagnosis

Throughout centuries, guessing about the sex of an unborn child has been a popular topic among expectant parents and their families. Until recently, fetal gender determination has been purely speculative. Improved resolution of ultrasound equipment has made it possible to visualize the fetal genitalia. Most studies are done beyond 20 weeks of gestation, when this information is often of sociologic interest only. Few studies have confined themselves to the early second trimester (16 to 20 weeks), when this information may have a medical impact, especially in cases of sex-linked diseases. The purpose of this communication is to report the results of a prospective study between 16 and 20 weeks, in which gender determination was assigned on the basis of sonographic criteria and confirmed by amniotic fluid karyotype results.

Subjects and methods

Our study population consisted of 115 patients with singleton pregnancies who were referred for genetic amniocentesis between 16 and 20 weeks' gestation. Gender determination was performed as a part of the sonographic examination preceding the amniocentesis procedure. The gestational age was based on the last menstrual period and its correlation with various sonographic measurements. The ultrasound machines used in this study were equipped with 3.0 or 3.5 mHz linear-array real-time transducers (Acuson 128, Acuson, Inc., Mountain View, California; General Electric RT 3000 and RT 2600, General Electric Medical Systems Div., Milwaukee, Wisconsin). All scans were obtained by two experienced sonographers. Fetal gender was prospectively assigned on the basis of imaging of the fetal external genitalia. This was accomplished by use of two planes of orientation, longitudinal and transverse views of the fetal pelvis, and with tilting of the transducer at appropriate angles to obtain the best image of the external genitalia. Male gender was assigned when the scrotum and penis were seen (Fig. 1). At this gestational age the testes are undescended and would not be visualized. Gender was designated as female if the labia majora were visualized (Fig. 2). Gender was not assigned when there was uncertainty. The final gender determination was based on the amniotic fluid karyotype results.

From the Section of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology at Yale University School of Medicine.

Received for publication September 12, 1986; accepted September 19, 1986.

Reprint requests: E. Albert Reece, M.D., Yale University School of Medicine, Department of Obstetrics and Gynecology, 333 Cedar St., New Haven, CT 06510.

Results

The results are summarized in Table I. Among 115 fetuses examined, fetal external genitalia were visualized with clarity for gender determination in 96 fetuses (83.5%). In 70 fetuses (60.9%) who were sonographically assigned as male, chromosome analysis confirmed 63 male fetuses (54.8%) and seven female fetuses (6.1%). Thus the true positive rate for male gender was 90%. All 26 fetuses assigned female by sonographic examination were confirmed to be female by chromosomal analysis. The overall accuracy rate for gender determination was 92.7%.

Comment

Knowledge of the fetal gender during the early second trimester is crucial in prenatal diagnosis and management of many medical conditions. In certain X-linked recessive disorders, the only relevant information obtained from a genetic amniocentesis is the fetal sex. This same information can be obtained by ultrasonography with a high degree of accuracy without the risk of pregnancy complications associated with

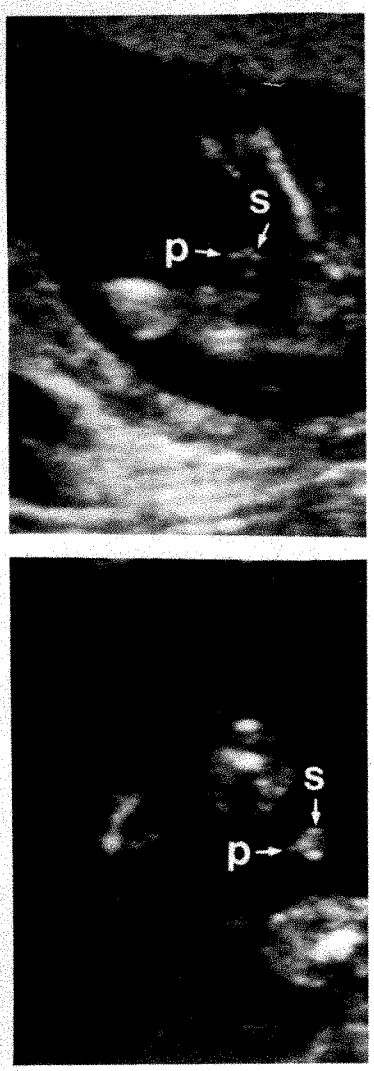


Fig. 1. The male external genitalia with penis *(p)* and scrotum *(s)* are demonstrated in a fetus of 18 weeks' gestation. *Upper panel* represents a cross-sectional view of the fetus at the level of the hip joint. With further tilting of the transducer the penis and scrotum are clearly seen *(lower panel)*.

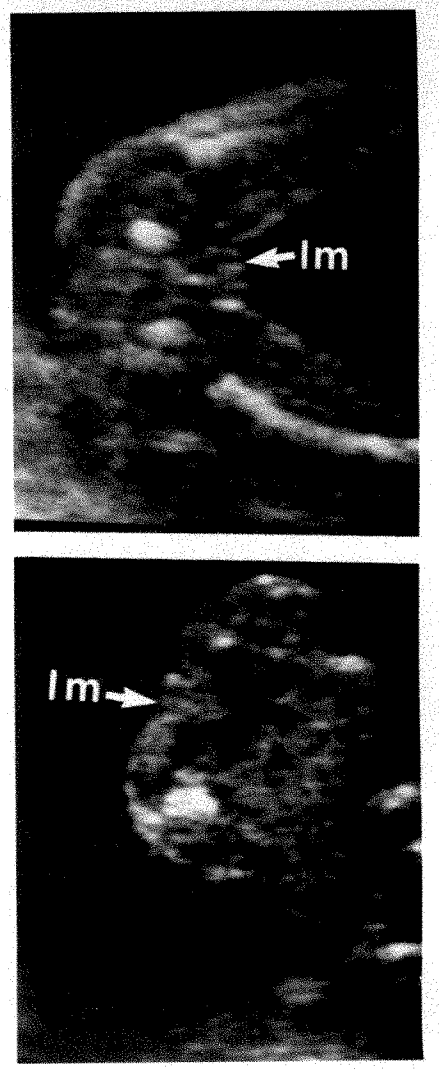


Fig. 2. Ultrasound picture demonstrating the female external genitalia in a fetus at 18 weeks of gestation. Labia majora *(lm)* were seen. *Upper panel* represents a transverse view of the pelvis at the level of the hip joint. *Lower panel* shows labia majora *(lm)* when the transducer was slightly tilted.

Table I. Gender determination in 115 fetuses between 16 and 20 weeks of gestation with the use of ultrasonography

Amniotic fluid karyotype	*Ultrasound gender determination*							
	Male		*Female*		*Not assigned*		*Total*	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
46,XY	63	54.8	0	—	11	9.6	74	64.4
46,XX	7	6.1	26	22.6	8	6.9	41	35.6
Total	70	60.9	26	22.6	19	16.5	115	100

True positive rates:

$$\text{Male fetuses} = \frac{63}{70} \times 100 = 90\%$$

$$\text{Female fetuses} = \frac{26}{26} \times 100 = 100\%$$

$$\text{Overall} = \frac{89}{115} \times 100 = 92.7\%$$

amniocentesis. Identification of a male fetus would prompt further study. In other sex-limited diseases such as posturethral valve obstruction, identification of a female fetus essentially rules out this disorder as a cause of fetal obstructive uropathy. In twin gestations with fetuses of different gender, ultrasonography can be useful in determining to which fetus a respective chromosome composition belongs.

In the gestational period between 16 and 20 weeks, rates of visualization of the fetal external genitalia have ranged from 15% to 40%, and error rates in those fetuses with sonographically visible external genitalia have ranged from 4% to 12%.[1] We were able to visualize the external fetal genitalia 83.5% of the time with a diagnostic accuracy rate of 92.7%. These results are comparable to those of Natsuyama,[2] in which he reported a 90% accuracy rate and visualization rates of 68% to 97%, depending on fetal position during the period of 15 to 19 weeks of gestation. His technique utilized multiple planes and sections as well as the identification of specific bony landmarks. He indicated that such complicated maneuvers would possibly be best suited for purely scientific interests. Our technique uses simple transverse and longitudinal planes, suited for routine ultrasound.

The results of our study demonstrate that gender determination during the early second trimester with the use of ultrasonography is possible with a high degree of accuracy. This information can be useful in the decision making and subsequent management of certain cases.

REFERENCES

1. Elejalde BR, Elejalde MM, Heitman T. Visualization of the fetal genitalia by ultrasonography. J Ultrasound Med 1980;4:633-9.
2. Natsuyama E. Sonographic determination of fetal sex from twelve weeks of gestation. Am J Obstet Gynecol 1984;149:748-57.

Atraumatic removal of secretions from the prepubertal vagina

Susan F. Pokorny, M.D., and Jane Stormer, L.V.N.
Houston, Texas

Adequate vaginal secretions for laboratory tests are difficult to obtain from the prepubertal girl. A simple atraumatic technique for obtaining several milliliters of vaginal secretions is described. This is extremely useful for the clinician evaluating the child with vulvovaginitis and/or possible sexual abuse. (Am J Obstet Gynecol 1987;156:581-2.)

Key words: Vaginal secretions, prepubertal, vulvovaginitis, sexual abuse

Anxiety is common among physicians who need to obtain secretions from the vagina of a prepubertal child. Because children rarely comply with these procedures, there is a chance of iatrogenic damage to the thin, delicate, unestrogenized mucosa of the genital tract. Furthermore, because these procedures can have long-lasting psychosocial effects, manipulation of the genital tract should be performed cautiously.

Techniques described in most textbooks for obtaining vaginal secretions are far from ideal.[1, 2] They can be traumatic and yield only small amounts of material. Q-tips are abrasive to the unestrogenized mucosa and should not be used. Eye droppers, pipettes, and plastic cannulas are smooth and unabrasive but can cause trauma secondary to inadvertent patient movement. Any single-chambered tube or cannula placed in a small vagina will be sucked against the vaginal wall when an effort is made to withdraw secretions, making the yield of material very small. Furthermore, the suction frequently alarms the child.

We have developed a very satisfactory atraumatic method for obtaining adequate vaginal secretions by use of a catheter-within-a-catheter. The outer catheter holds the vaginal wall away from the inner catheter, through which the diluting solution is injected and withdrawn from the vagina. The two catheters are obtained by cutting (with a sterile technique) 4 inches from the distal end of a No. 12 bladder catheter and the proximal (hub end) 4½ inches of an intravenous butterfly catheter. The butterfly tubing is inserted inside the bladder catheter and a small syringe with approximately 1 ml of sterile normal saline solution is attached

From the Departments of Obstetrics and Gynecology and Pediatrics, Baylor College of Medicine.
Received for publication July 29, 1986; accepted August 29, 1986.
Reprint requests: Susan F. Pokorny, M.D., Department of Obstetrics and Gynecology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

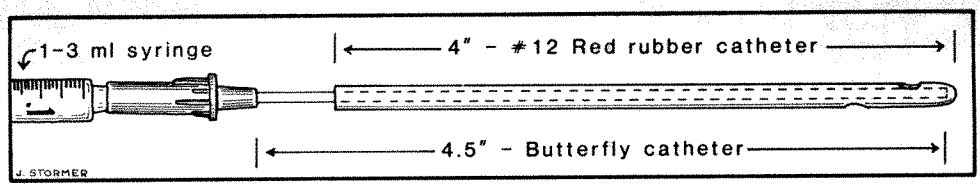


Fig. 1. Assembled catheter-within-a-catheter, as used to obtain samples of vaginal secretions from prepubertal patients.

to the hub of the "butterfly" tubing (Fig. 1). This makes a catheter-within-a-catheter that can be gently inserted into the vagina of the child. Should the child move suddenly, the examiner can brace the insertion hand against the thigh of the child and the catheters remain in place. Although some children report that they "feel" the insertion, once the catheter is in place, none have reported a painful experience.

Once the catheter is inserted, the examiner can alternately inject and aspirate the small amount of diluting fluid a few times to obtain a good admixture of material from the upper vagina and then quickly withdraw the whole apparatus after drawing the material back into the syringe. Depending on the situation, the examiner can obtain from one atraumatic procedure enough fluid for the following: an examination for sperm particles and acid phosphatase, a Gram stain, a gonorrheal culture, a chlamydial culture, a "wet mount," and other cultures as needed.

Many children with vulvovaginitis go through needless courses of creams and antibiotics because the infectious organism is never identified. Many children who have been sexually exploited have their stories unsubstantiated because material for forensic rape kits and/or cultures is never obtained. It is hoped through use of the technique described in this paper that the management of children with these common pediatric gynecology problems will be improved.

REFERENCES

1. Altcheck A. Pediatric vulvovaginitis. J Reprod Med 1984; 29:359-75.
2. Emans SJ, Goldstein DP. Pediatric and adolescent gynecology. Boston: Little, Brown, 1982:47.

Preliminary report on the use of tamoxifen in the treatment of endometriosis

G. M. Haber, M.D., and Y. F. Behelak, Ph.D., M.D.
Montreal, Quebec, Canada

Tamoxifen, an antiestrogen, was used in the treatment of two women with a long-standing history of endometriosis. After a 6-month period of treatment, both patients were symptom free and both showed a marked decrease in the size of their endometriotic lesions. It was significant that ovulation was documented in both patients while receiving the treatment regimen. The use of tamoxifen as an alternative modality in the treatment of endometriosis, especially for women desiring to conceive, is discussed. (Am J Obstet Gynecol 1987;156:582-6.)

Key words: Tamoxifen, endometriosis, treatment

Tamoxifen, as well as other related antiestrogen compounds, seems to have some estrogen-like effects and, like estrogens, underwent translocation from the cytoplasm to the nucleus. However, in contrast to 17β-estradiol, the antiestrogen receptor complex exhibited long-term nuclear retention resulting in early estrogen-like responses but a later manifestation of estrogen antagonism by failing to replenish the cytoplasmic estrogen receptors.

In this preliminary study we report on the use of the antiestrogen tamoxifen in the treatment of two cases of endometriosis which were unresponsive to the usual

From the Departments of Obstetrics and Gynecology, The Montreal General Hospital and St. Mary's Hospital, McGill University.
Received for publication March 27, 1986; revised August 25, 1986; accepted October 8, 1986.
Reprint requests: Dr. G. M. Haber, M.D., Montreal General Hospital, 1650 Cedar Ave., Montreal, Quebec, Canada H3G 1A4.

therapeutic modalities currently in use such as danazol or the estrogen/progestin combination oral contraceptive.

Case reports

Case 1. This patient came to medical attention when she presented at age 29 with signs and symptoms of an acute abdomen. At laparotomy she was found to have a ruptured endometrial cyst of the right ovary. She had endometrial lesions on the sigmoid colon, with adhesion of the rectosigmoid to the posterior cul-de-sac. The left ovary had a small 2 cm cyst, while both tubes appeared normal.

On discharge from hospital she was to receive 200 mg of danazol three times daily. She continued the administration of danazol for 3 months, after which treatment was switched to an estrogen/progestin combination oral contraceptive, which she received for a period of 6 months.

The patient was next seen 9 months later with a history of reactivation of endometriosis, especially bowel symptoms. She was restarted on a 9-month course of danazol, at the end of which she underwent a diagnostic laparoscopy and laparotomy. Findings at laparotomy showed dense adhesions throughout the pelvis. Bilateral salpingoovarioplasty and lysis of adhesions were carried out, and the patient was observed over a period of 1 year during which time she tried unsuccessfully to conceive. Meanwhile her symptoms worsened rapidly with clinical evidence of significant uterosacral ligament thickening.

In view of the extreme pain she was enduring which was not responsive to analgesics or antiprostaglandins, tamoxifen therapy was suggested to her in lieu of the radical surgical treatment that she was now insistently demanding. She was started on a protocol of 10 mg of tamoxifen (ICI Pharma) twice daily for a clinical trial period of 6 months, to be followed by a diagnostic laparoscopy.

Case 2. This patient was seen at age 24 with a 6-year history of severe dysmenorrhea and irregular intermenstrual bleeding. She had been receiving oral contraceptives intermittently for a period of 8 years. Her physical examination at the time was within normal limits, and the oral contraceptives were continued. Two years later she opted to stop using the oral contraceptives and tried unsuccessfully to conceive in spite of a negative infertility workup. She now complained of increasing pelvic pain, dyspareunia, and dysmenorrhea. Clinical examination revealed prominant nodularity of her uterosacral ligamants.

Laparoscopy revealed scattered endometriotic lesions in the posterior cul-de-sac and on the uterosacral ligaments. The tubes seemed free of lesions but were adherent and showed some degree of obstruction with hydrotubation. The ovaries had several small endometriotic lesions.

The patient was next treated with danazol for a period of 8 months, which was followed by a recurrence of her symptoms after discontinuation of the drug. A second 6-month course of danazol failed to improve her condition, and she still failed to conceive. After discussion with the patient, tamoxifen therapy was suggested, and she agreed to a 6-month trial period to be followed by a laparoscopy.

Serial determinations of serum levels of follicle-stimulating hormone, luteinizing hormone, and progesterone were carried out during the midluteal phase of the patients' menstrual cycles both before and after treatment with tamoxifen. Concurrent with the treatment, the patients were asked to keep a diary, recording any symptoms they incurred during the treatment period. Both patients were seen and examined at 6-week intervals for subjective and objective evaluation of their condition and for documentation of any clinical signs of improvement. Patient A recorded her basal body temperatures throughout the study period. At the end of a 6-month tamoxifen regimen both patients underwent a diagnostic laparoscopy.

Results

The hormonal profile documented over the 6-month study period was as follows: The levels of serum follicle-stimulating hormone, luteinizing hormone, and progesterone were as expected for day 19 of the control or pretreatment cycle. Treatment with tamoxifen did not result in significant changes in the levels of any of the hormones assayed. Progesterone levels at midluteal phase were consistent with ovulation throughout the 6-month treatment period.

Subjectively both patients complained of hot flushes, which were more frequent early in the treatment period. They both also noted increased fatigue and constipation. One of the patients noted an exacerbation of acne. It is significant that they both noted that all of their symptoms were relatively less pronounced than during their treatment with danazol, and neither patient complained of breast tenderness. Menses was normal and no voice change or hair loss was noted. Both were symptom free 6 months after treatment.

Clinical examination of Patient 1 showed no improvement after 6 weeks of treatment with the thickening of the uterosacral ligaments quite prominent and tender. By 12 weeks, however, there was less tenderness and by 6 months there was minimal clinical evidence of cul-de-sac scarring.

Patient 2 showed marked improvement during treatment but the symptoms recurred, albeit to a lesser degree, 3 to 4 months following discontinuation of treatment with tamoxifen.

Laparoscopic examination at the end of the treatment period was very rewarding. We found very little evidence of endometriotic lesions except for an occasional trace of inactive endometriosis.

Neither of the patients became pregnant, although both did document regular ovulation while receiving tamoxifen therapy.

Comment

Antiestrogens are nonsteroidal compounds capable of antagonizing many of the actions of estrogens. In particular, these compounds have been shown to suppress estrogen-stimulated uterine growth in a variety of animal species and to cause the regression of hormone-dependent mammary tumors as well as to inhibit the growth of estrogen receptor containing human breast cancer cells.[1-4] They also tend to alter gonadotrophin secretion patterns in premenopausal women.[5]

The study of these compounds was initially prompted by the search for an effective contraceptive agent; however, they are now viewed with more interest for their capacity to control the growth of hormone-dependent neoplasms, particularly tumors of the breasts and more recently for the induction of ovulation in women with luteal phase deficiency.[6,7]

The hormone responses to short-term administration of tamoxifen for induction of ovulation have been studied by a number of authors.[5,8-11] Groom and Griffiths[11] observed a twofold to eightfold increase in estradiol levels during the test cycles, which was most pronounced at the times of midcycle and midluteal peaks.

The plasma concentrations of luteinizing hormone did not vary from control levels and those reported for normal ovulatory women.[12] There was a slight increase observed in plasma progesterone concentrations after tamoxifen treatment.[11]

Similar results were obtained by Sherman et al.[5] who studied the effects of long-term administration of tamoxifen in premenopausal women. In their report all subjects continued to have regular menses with biphasic basal body temperature records. The mean estradiol concentration at the midcycle peak and during the luteal phase was twice that observed during the nontreatment cycle. They also observed that the concentration and secretory patterns of luteinizing hormone and follicle-stimulating hormone were not greatly changed. The integrated luteal phase progesterone concentrations during tamoxifen cycles were increased over those of control cycles although the secretion pattern observed was similar to that in nontreated women.

In their study of tamoxifen as a treatment for luteal phase deficiency Fukushima et al.[9] found 10 of 12 endometrial specimens obtained during the midluteal phase of tamoxifen cycles to have a maturation delay of 2 or more days in spite of normal levels of progestin and estradiol concentrations.

Using treatment with tamoxifen (10 mg daily for 5 days) in patients with ovulatory menometrorrhagia, El-Sheikha et al.[13] reported that the appearance of the endometrium during the luteal phase of the treated cycle was marked by a pseudodecidual reaction with a disproportionate effect on the stroma, suggestive of an inadequately prepared endometrium by estrogen. Similar results were obtained by Fukushima et al.[9] In a study of the effect of tamoxifen and medroxyprogesterone acetate on human endometrial estrogen and progesterone receptor concentrations, Kokko et al.[14] noted a decreasing trend of both estrogen and progestin cytosol receptor concentrations but little effect on nuclear estrogen and progestin receptors. In the same study the administration of medroxyprogesterone acetate together with tamoxifen significantly increased the concentration of endometrial nuclear estrogen receptor over that of control endometrium taken on day 23 of the cycle. They concluded that tamoxifen may inhibit the estrogen-mediated synthesis of progesterone receptors in the endometrium during the early secretory phase of the menstrual cycle, resulting in delayed endometrial maturation despite normal progesterone and estradiol concentrations. In spite of these findings, Fukushima et al.[9] reported successful pregnancies in six of 17 patients treated with a short-term tamoxifen regimen (10 mg daily for 5 days).

Coutinho[15] in 1982 reported on the use of a synthetic antiestrogen/antiprogesterone compound, gestrinone, for the treatment of endometriosis. The compound, a synthetic trienic 19-steroid has an affinity for progesterone receptors and an antiestrogenic effect in spite of the fact that it does not bind estrogen receptors. It was given in a dose of 5 mg twice weekly to women with endometriosis, and the author noted that all the subjects were symptomless by the end of the third month of treatment. These patients, however, became amenorrheic at the end of the second month of treatment. In the same study five of nine women who had the potential and desire to conceive did so over a period of three to 14 months after the discontinuation of the drug.

Tamoxifen was used for the treatment of two cases of endometrial stromal tumors which were positive for estrogen receptors in the tumor tissue.[16] The authors, however, report a deterioration of the patients' conditions with progression of their tumors.

In our study the administration of tamoxifen (10 mg twice daily) did not seem to alter the secretory patterns of luteinizing hormone and follicle-stimulating hormone; moreover, the concentrations of progesterone at or around day 20 of the treatment cycles indicated ovulation during tamoxifen therapy. These results are in agreement with those of Sherman et al.[5] who studied the effect of long-term administration of tamoxifen to premenopausal women.

Although neither of the patients did conceive, both were relatively symptom free during the treatment period, and laparoscopic evidence indicated a complete regression of their endometriotic lesions after 6 months of treatment with tamoxifen.

The fact that the two patients ovulated and thus could

potentially conceive while receiving treatment raises the problem of teratogenicity in the fetus. A careful search of the literature showed a paucity of reports concerning the teratogenic effects of tamoxifen in humans. In rats, Chamness et al.[17] have shown that tamoxifen administered to newborn female animals caused abnormalities in reproductive development. These authors found that vaginal opening occurred early, cycles were absent, and at 4 months of age all ovaries and uteri were atrophic while oviducts showed severe metaplasia with abscess formation.

Similar observations, also in the rat, were made by Clark et al.[18] who noted hypertrophied and hyperplastic oviducts; ovarian, oviductual, and uterine inflammation accompanied by pyometra; and, in some animals, atrophic uteri and ovaries.

Taguchi and Nishizuka[19] treated neonatal mice with a relatively high dose of tamoxifen and have noted that all treated mice developed reproductive tract anomalies. These were characterized by an abnormal urethral opening into the vaginal lumen and a significant reduction in the size of the common cervical canal. In addition, these mice developed evidence of uterine hypoplasia and absence of corpora lutea.

In a recent communication Diver et al.[20] reported on nonspecific congenital abnormalities in fetuses inadvertently exposed to tamoxifen as well as on healthy babies born to patients receiving tamoxifen. They also report that tamoxifen terminates pregnancies in rats and rabbits; however, in doses that do not do so, no teratogenic effects were found. Deformation or rib cage and altered ossification patterns in the rat were ascribed to uterine pressure effects resulting from inhibition of the estrogenic effect on the pregnant uterus. These multiple deformities were found to disappear after birth.[20]

In our review of the literature we found no reports that tamoxifen is associated with an increase in the incidence of any malignancy in humans. Authors who have used tamoxifen to induce ovulation with a successful pregnancy outcome have not reported on any fetal abnormalities. It must be remembered, however, that these authors used tamoxifen for a short term only, and a number of pregnancies did end in early abortions.[9,21]

It is our conclusion that the use of tamoxifen for the treatment of endometriosis may be useful in a number of situations: (1) Tamoxifen offers symptomatic relief of pain with minimal side effects compared to danazol treatment. (2) It could certainly be used to reduce endometriotic tumors and lesions before definitive surgical treatment. (3) It is relatively inexpensive. (4) Since tamoxifen does not seem to inhibit ovulation, its administration to women suffering from infertility secondary to endometriosis may help in allowing these women to conceive. However, further studies are required to determine its teratogenic effect, if any, on the human fetus before it could be used on a large scale for this purpose.

In view of this lack of knowledge regarding teratogenicity, we have modified our protocol in subsequent trials in women with the potential risk of conceiving while receiving treatment (Behelak and Haber, manuscript in preparation) while simultaneously continuing studies on women not at risk for pregnancy to determine the efficacy of the drug when used intermittently, or with varying doses, to treat lesser grades of endometriotic lesions.

REFERENCES

1. DeSombre E, Green G, Jensen E. Estrophilin and endocrine responsiveness of breast cancer. Prog Cancer Res Ther 1978;10:1.
2. Howitz K, McGuire W. Antiestrogens: mechanisms of action and effects in breast cancer. In: McGuire W, ed. Breast cancer advances in research and treatment. vol. 2. New York: Plenum, 1978;155-205.
3. Sutherland R, Jordon V. Nonsteroidal antiestrogens. New York: Academic Press, 1981.
4. Lippman M, Bolan G, Huff K. The effects of estrogens and antiestrogens on hormone-responsive human breast cancer in long term tissue cultures. Cancer Res 1976; 36:4595.
5. Sherman B, Chapler R, Crickard K, Wycoff D. Endocrine consequences of continuous antiestrogen therapy with tamoxifen in premenopausal women. J Clin Invest 1979; 64:398.
6. Klopper A, Hall M. New synthetic agent for the induction of ovulation; preliminary trials in women. Br Med J 1971;1:152.
7. Williamson J, Ellis J. The induction of ovulation by tamoxifen. Br J Obstet Gynecol 1973;80:844.
8. Tajima C. Luteotropic effects of tamoxifen in infertile women. Fertil Steril 1984;42:223.
9. Fukushima T, Tajima C, Fukam K, Maeyama M. Tamoxifen in the treatment of infertility associated with luteal phase deficiency. Fertil Steril 1982;37:755.
10. Tajima C, Fukushima T. Endocrine profiles in tamoxifen induced ovulatory cycles. Fertil Steril 1983;40:23.
11. Groom F, Griffiths K. Effect of the antiestrogen tamoxifen on plasma levels of luteinizing hormone, follicle stimulating hormone, prolactin, estradiol and progesterone in normal premenopausal women. J Endocrinol 1976; 70:421.
12. Speroff L, Glass R, Kase N. Clinical gynecologic endocrinology and infertility. 3rd ed. Baltimore: Williams & Wilkins. 1983.
13. El-Sheikha A, Klopper A, Beck J. Treatment of menometrorrhagia with an antiestrogen. Clin Endocrinol 1972; 1:275.
14. Kokko E, Janne O, Kauppila A, Vihko R. Effects of tamoxifen, medroxyprogesterone acetate, and their combination on human endometrial estrogen and progestin receptor concentrations, 17-hydroxysteroid dehydrogenase activity, and serum hormone concentrations. Am J Obstet Gynecol 1982;143:382.
15. Coutinho E. Treatment of endometriosis with gestrinone (R-2323), a synthetic antiestrogen, antiprogesterone. Am J Obstet Gynecol 1982;144:895.
16. Brons J, Lene K, Rasmussen J. Hormone treatment of stromal endometriosis. Acta Obstet Gynecol Scand 1980; 59:471.
17. Chamness G, Bannayan G, Landry L, Sheridan P, McGuire W. Abnormal reproductive development in rats after neonatally administered antiestrogen/tamoxifen. Biol Reprod 1979;21:1087.

18. Clark J, Guthrie S, McCormack S. Neonatal stimulation of the uterus by clomiphene, tamoxifen and nafoxidine: relationship to the development of reproductive tract abnormalities. Adv Exp Med Biol 1981;138:87.
19. Taguchi O, Nishizuka Y. Reproductive tract abnormalities in female mice treated neonatally with tamoxifen. AM J OBSTET GYNECOL 1985;151:675.
20. Diver J, Jackson I, Fitzgerald J. Tamoxifen and nonmalignant indications. Lancet 1986;2:733.
21. Tsuiki A, Uehara S, Kyono K, et al. Induction of ovulation with an estrogen antagonist tamoxifen. Tohoku J Exp Med 1984;144:21.

Exaggerated gonadotropin response to luteinizing hormone–releasing hormone in amenorrheic runners

Janet Yahiro, Ph.D., Allan R. Glass, M.D., William B. Fears, M.D., Earl W. Ferguson, M.D., Ph.D., and Robert A. Vigersky, M.D.
Bethesda, Maryland, and Washington, D. C.

Most studies of exercise-induced amenorrhea have compared amenorrheic athletes (usually runners) with sedentary control subjects. Such comparisons will identify hormonal changes that develop as a result of exercise training but cannot determine which of these changes play a role in causing amenorrhea. To obviate this problem, we assessed reproductive hormone status in a group of five amenorrheic runners and compared them to a group of six eumenorrheic runners matched for body fatness, training intensity, and exercise performance. Compared to the eumenorrheic runners, the amenorrheic runners had lower serum estradiol concentrations, similar basal serum luteinizing hormone and follicle-stimulating hormone concentrations, and exaggerated responses of serum gonadotropins after administration of luteinizing hormone–releasing hormone (100 μg intravenous bolus). Serum prolactin levels, both basally and after thyrotropin-releasing hormone administration (500 μg intravenous bolus) or treadmill exercise, was similar in the two groups, as were serum thyroid function tests (including thyrotropin response to thyrotropin-releasing hormone). Changes in serum cortisol levels after short-term treadmill exercise were similar in both groups, and serum testosterone levels increased after exercise only in the eumenorrheic group. In neither group did such exercise change serum luteinizing hormone, follicle-stimulating hormone, or thyrotropin levels. We conclude that exercise-induced amenorrhea is not solely related to the development of increased prolactin output after exercise training. The exaggerated gonadotropin response to luteinizing hormone–releasing hormone seen in amenorrheic runners in comparison with matched eumenorrheic runners is consistent with a hypothalamic etiology for the menstrual dysfunction, analogous to that previously described in "stress-induced" or "psychogenic" amenorrhea. (AM J OBSTET GYNECOL 1987;156:586-91.)

Key words: Exercise, amenorrhea, luteinizing hormone–releasing hormone

Secondary amenorrhea is common in women who exercise intensively, such as long-distance runners and ballet dancers, but the mechanism of exercise-associated menstrual irregularity has not been conclusively established. Etiologic factors that have been suggested as having significance in this disorder include a decrease in body fatness, an "energy drain" caused by increased energy output, augmented prolactin release following exercise, hyperandrogenism, excess psychological stress, and increased central nervous system β-endorphin activity. The differences in conclusions among these studies may reflect in part the differences in subject selection and the lack of an appropriately matched control group. The purpose of the current study was to determine the hormonal abnormalities specifically associated with exercise-induced amenorrhea. To do this we compared amenorrheic runners to a control group of eumenorrheic runners who were matched for age, percent of body fat, training intensity,

From the Departments of Physiology and Medicine, Uniformed Services University of Health Sciences, and the Departments of Medicine and Clinical Investigation, Walter Reed Army Medical Center.
The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the Department of the Army, Department of the Air Force, or the Department of Defense.
Received for publication May 5, 1986; revised September 29, 1986; accepted October 6, 1986.
Reprint requests: Allan R. Glass, M.D., Endocrinology Service, 7D, Walter Reed Army Medical Center, Washington, DC 20307.

Table I. Anthropometric and exercise data on women runners*

	Eumenorrheic	*Amenorrheic*
Age (yr)	28.8 ± 4.6	29.4 ± 6.1
Body fat (%)	15.9 ± 6.6	12.2 ± 5.7
Training intensity (miles/wk)	51.8 ± 18.8	46.3 ± 19.7
Training performance (min/mile)	7.7 ± 0.5	8.7 ± 0.8
Time on treadmill (min)	14.6 ± 2.3	13.2 ± 0.8
Oxygen consumption (ml/kg · min)		
Resting	3.0 ± 0.8	2.1 ± 0.9
Maximal during exercise	52.9 ± 6.0	46.2 ± 4.2

*Mean ± SEM. No statistical difference was observed between the two groups for any parameter.

and level of fitness (maximum oxygen consumption). By contrast, previous studies using sedentary women as controls have not been able to distinguish which of the hormonal changes related to exercise training might be important in leading to amenorrhea.

Methods

Subjects. Six eumenorrheic runners (6 months or more of regular menses) and five amenorrheic runners (6 months or more without menses) were recruited from various running clubs based in Washington, D.C. The groups were matched as closely as possible for age, fatness, and running ability (Table I). Four of five amenorrheic runners were nulliparous as compared to three of six eumenorrheic runners. Although none of the runners was amenorrheic before acquiring an interest in running, two of six eumenorrheic runners had a history of oligomenorrhea in the distant past, compared to four of five amenorrheic runners. One amenorrheic runner reported that menses would resume if she dropped her weekly mileage below 40 miles per week, but she had not done so in over 2 years. None of the subjects had taken oral contraceptives for at least 2 years, and no subject took any medication regularly. All eumenorrheic runners were tested in the early follicular phase of their menstrual cycle (days 2 to 7). All women were instructed to fast for 12 hours and refrain from exercise for 24 hours before testing. Informed consent was obtained before all studies, and this study was approved by our institutional review board.

Exercise study. Each runner exercised on a treadmill using the Bruce protocol to maximum oxygen consumption as determined by a Beckman Metabolic Measurement Cart (Beckman Instrument Inc., 3900 River Road, Schiller Park, IL 60176). Before the exercise period, subjects were allowed to relax in an armchair while control blood samples were obtained at −30 and 0 minutes. Oxygen consumption, carbon dioxide production, and electrocardiographic changes were monitored continuously during exercise, and blood samples were obtained at 0, 15, 30, 45, and 60 minutes after completion of exercise. Basal and maximal oxygen consumption were similar in the amenorrheic and eumenorrheic groups (Table I). Blood was allowed to clot at room temperature, and the serum was frozen at −20° C until analysis. Each sample was analyzed by radioimmunoassay for luteinizing hormone, follicle-stimulating hormone, thyroid-stimulating hormone, prolactin, testosterone, and cortisol. All samples were measured in the same assay run. Intra-assay variation was <10% for all assays. Postexercise hormone values were corrected for changes in plasma volume. Percent of body fat determinations were obtained by use of the skin fold thickness method.

Luteinizing hormone–releasing hormone/thyrotropin-releasing hormone test. On the morning after the exercise study, serum luteinizing hormone, follicle-stimulating hormone, thyroid-stimulating hormone, and prolactin were measured at −30, −15, 0, 15, 30, 45, 60, 90, and 120 minutes after the administration of luteinizing hormone–releasing hormone (100 μg) and thyrotropin-releasing hormone (500 μg) given as a single intravenous bolus. Serum estradiol, serum progesterone, and serum thyroxine levels and T_3 resin uptake were also measured on basal samples.

Data analysis. Basal hormone levels and the responses to exercise, luteinizing hormone–releasing hormone, and thyrotropin-releasing hormone in the study groups were compared by the Wilcoxon rank sum test.

Results

Basal serum luteinizing hormone, follicle-stimulating hormone, testosterone, progesterone, and cortisol concentrations were similar in the amenorrheic and eumenorrheic runners, while serum estradiol level was lower in the amenorrheic group (Table II). The response of serum luteinizing hormone and follicle-stimulating hormone to luteinizing hormone–releasing hormone (100 μg) was assessed by both the peak gonadotropin level attained as well as the area under the curve. The luteinizing hormone area under the curve after luteinizing hormone–releasing hormone was greater in the amenorrheic group (Table II), and although there was more variability in the amenorrheic group (range of responses, 2958 to 10225 mIU ·

Table II. Hormone levels in exercise-induced amenorrhea*

	Eumenorrheic	*Amenorrheic*
Basal levels		
Serum luteinizing hormone (mIU/ml)	12.1 ± 2.2	11.9 ± 3.7
Serum follicle-stimulating hormone (mIU/ml)	14.3 ± 2.6	17.5 ± 1.4
Serum testosterone (ng/dl)	28.7 ± 3.4	25.9 ± 4.0
Serum cortisol (μg/dl)	12.6 ± 8.5	14.0 ± 1.7
Serum progesterone (ng/ml)	0.25 ± 0.06	0.14 ± 0.09
Serum estradiol (pg/ml)	61.5 ± 18.6	22.0 ± 6.9†
Response to luteinizing hormone–releasing hormone		
Luteinizing hormone		
Peak level (mIU/ml)	37.9 ± 6.5	70.0 ± 20.9
Area under curve (mIU · min/ml)	2315 ± 188	5129 ± 1184†
Follicle-stimulating hormone		
Peak level (mIU/ml)	26.0 ± 2.5	36.3 ± 2.5†
Area under curve (mIU · min/ml)	809 ± 288	1593 ± 207

*Mean ± SEM.
†$p < 0.01$ vs. eumenorrheic.

Table III. Prolactin and thyroid function in exercise-induced amenorrhea*

	Eumenorrheic	*Amenorrheic*
Serum prolactin (ng/ml)		
Basal	14.1 ± 1.3	10.9 ± 0.8
Peak after thyrotropin-releasing hormone	70.7 ± 13.0	77.5 ± 21.0
Peak after exercise	27.1 ± 4.3	21.2 ± 6.9
Serum thyroxine (μg/dl)	7.3 ± 0.3	7.3 ± 0.6
Serum triiodothyronine (ng/dl)	126 ± 2	125 ± 1
Serum thyrotropin (μU/ml)		
Basal	4.3 ± 1.3	3.1 ± 0.2
Peak after thyrotropin-releasing hormone	14.2 ± 1.4	22.9 ± 4.0

*Mean ± SEM.

min/ml; range in eumenorrheics 1581 to 3025 mIU · min/ml), all but one of the amenorrheic group had a response greater than the highest value seen in the eumenorrheic group. The luteinizing hormone peak level also appeared greater in the amenorrheic group, but this difference did not attain statistical significance. Similarly, the peak follicle-stimulating hormone level after luteinizing hormone–releasing hormone was greater in the amenorrheic group (Table II), but the follicle-stimulating hormone area under the curve was not statistically different in the two groups.

Basal serum prolactin and the prolactin response to thyrotropin-releasing hormone administration (500 μg) were not different in the amenorrheic and eumenorrheic runners (Table III). Similarly, the exercise-induced rise in serum prolactin was also not different in the two groups (Table III). Thyroid function tests, including the thyroid-stimulating hormone to thyrotropin-releasing hormone, were comparable in the two groups (Table III). Postexercise serum levels of luteinizing hormone, follicle-stimulating hormone, thyroid-stimulating hormone, testosterone, and cortisol were the same in the amenorrheic and eumenorrheic groups (Table IV); cortisol showed a significant rise with exercise in both groups, while serum testosterone was significantly increased over preexercise values only in the eumenorrheic group.

Comment

With the rising interest of American women in exercise programs, the incidence of exercise-related amenorrhea has increased over the past several years. Despite intensive study in recent times, the mechanism of amenorrhea that develops during heavy aerobic exercise remains unestablished. Multiple factors, both hormonal (hyperprolactinemia, androgen excess) and nonhormonal (weight loss, low body fat, energy drain, stress) have been proposed as having an etiologic role in exercise-induced amenorrhea. To establish the role of a hormonal or other abnormality in causing runner's amenorrhea requires a comparison of amenorrheic and nonamenorrheic runners in order to exclude those changes resulting from exercise training which are unrelated to the development of amenorrhea. This type of comparison is also needed to settle a major unanswered question in this area: Does the development of

Table IV. Peak hormone levels after treadmill exercise*

	Eumenorrheic	*Amenorrheic*
Serum luteinizing hormone (mIU/ml)	14.1 ± 1.1	20.2 ± 6.9
Serum follicle-stimulating hormone (mIU/ml)	18.5 ± 0.3	23.3 ± 2.2
Serum cortisol (μg/dl)	18.2 ± 1.8	21.1 ± 3.4
Serum thyroid-stimulating hormone (μU/ml)	3.7 ± 0.2	3.7 ± 0.2
Serum testosterone (ng/dl)	36.1 ± 3.8	29.9 ± 6.5

*Mean ± SEM. All values corrected for plasma volume changes.

exercise-induced amenorrhea in some women reflect an abnormal hormonal response to exercise training which is seen only in those amenorrheic women, or does exercise training produce a similar hormonal alteration in all women, with amenorrhea developing only in those with preexisting abnormalities of endocrine function? For example, the suggestion that amenorrheic runners have a higher incidence of menstrual abnormalities before beginning their training supports the concept of a population subset that is peculiarly sensitive and predisposed to exercise-induced amenorrhea. However, the ability of increased exercise training to cause menstrual abnormalities in an otherwise unselected group suggests that prior abnormality of pituitary-ovarian function may not be necessary for development of exercise-induced amenorrhea.[1]

Hyperprolactinemia is a well-recognized cause of amenorrhea. The finding that female runners showed a rise in serum prolactin after acute exercise, but sedentary women did not,[2] raised the possibility that amenorrhea in women runners occurred because they developed the ability to increase serum prolactin after exercise, with menstrual disruption resulting from this intermittent hyperprolactinemia. Such a role for increased prolactin output was supported by the finding that thyrotropin-releasing hormone-induced prolactin release increased as women increased the intensity of exercise.[3] However, comparisons of amenorrheic and eumenorrheic runners showed that the prolactin response to acute exercise was not greater in the amenorrheic group[2, 4] and amenorrheic and eumenorrheic runners did not differ in basal serum prolactin.[2] Likewise, our study showed no difference between amenorrheic and eumenorrheic runners in basal serum prolactin or in the serum prolactin response to acute exercise or to thyrotropin-releasing hormone. Thus, although increased prolactin output may be a consequence of exercise training, the similarity of prolactin response in amenorrheic and eumenorrheic runners suggests that hyperprolactinemia by itself does not cause runner's amenorrhea. We cannot exclude the possibility that the increased prolactin output related to exercise training may induce amenorrhea in a subset of the population with increased sensitivity to prolactin's effects on menstrual function.

Androgen excess is associated with menstrual abnormalities under a variety of circumstances, such as polycystic ovarian disease. Initial findings of elevated basal serum testosterone or exercise-induced increases in serum testosterone in amenorrheic runners raised the possibility that transient or persistent hyperandrogenism might cause the amenorrhea. However, exercise results in hemoconcentration, and in our study there was no change in serum testosterone after exercise in amenorrheic runners after correction for changes in plasma volume, although such an exercise-induced increase was noted in eumenorrheic runners. Others have failed to find exercise-induced increases in serum testosterone in amenorrheic runners,[4] and neither we nor others[4] have found increases in basal serum testosterone concentrations in this group.

Much attention has been focused on gonadotropin levels in women with exercise-induced amenorrhea. In comparison with sedentary women, amenorrheic runners have generally been found to have normal[2, 5, 6] or reduced[4, 7] basal serum gonadotropin levels. Likewise, in two previous studies in which nonamenorrheic runners, studied in the late follicular or late luteal phases, were compared to sedentary controls, reductions were observed in the gonadotropin response to luteinizing hormone–releasing hormone.[5, 8] By contrast, in our study, the only one in which responses in amenorrheic runners were compared to those in eumenorrheic runners matched for fatness and fitness (studied in the early follicular phase), the amenorrheic runners had greater gonadotropin responses to a large bolus dose of luteinizing hormone–releasing hormone than did the eumenorrheic runners. Similarly, gonadotropin responses to lower doses of luteinizing hormone–releasing hormone in severely oligoamenorrheic runners were also greater than in sedentary controls,[6] and an earlier report also described one amenorrheic runner with an exaggerated gonadotropin response to luteinizing hormone–releasing hormone.[7] Such hyperresponses to luteinizing hormone–releasing hormone are unlikely to be due to primary ovarian failure, since basal serum gonadotropins were not elevated and estradiol responses to luteinizing hormone–releasing hormone were reported to be normal.[6] These exaggerated gonadotropin responses to luteinizing hormone–releas-

ing hormone are similar to those reported previously in "hypothalamic,"[9] "psychogenic,"[10] or "stress"[11] amenorrhea, although previous studies have differed on whether amenorrheic runners are subject to an unusual amount of stress. In contrast, the gonadotropin responses to luteinizing hormone–releasing hormone in amenorrhea associated with weight loss, although variable, are often reduced. The finding of hormonal differences between eumenorrheic and amenorrheic runners may suggest that the latter have an idiosyncratic hormonal response to exercise training rather than simply having an underlying hypothalamic-pituitary-ovarian axis which is more vulnerable to perturbation by the exercise-induced hormonal changes occurring in all women.

Pituitary output of gonadotropin depends on the frequency and magnitude of pulsatile luteinizing hormone–releasing hormone output from the hypothalamus as well as the pituitary sensitivity to luteinizing hormone–releasing hormone. The latter, in turn, depends strongly on the level of chronic luteinizing hormone–releasing hormone release (the self-priming effect of luteinizing hormone–releasing hormone) as well as on gonadal steroids, such as estradiol, which can have both positive and negative feedback effects on pituitary gonadotropin output. Several aspects of this scheme have been studied in amenorrheic runners. In particular, recent emphasis has been placed on the findings that amenorrheic runners have reduced frequency of luteinizing hormone–releasing hormone secretion[6] and that acute exercise can reduce the frequency of luteinizing hormone–releasing hormone secretion.[12] However, since eumenorrheic runners also have reduced frequency of pulsatile luteinizing hormone–releasing hormone secretion[13] and some amenorrheic runners have normal rates for pulsatile luteinizing hormone–releasing hormone secretion,[6] it appears that reduced frequency of luteinizing hormone–releasing hormone secretion alone may not be a sufficient explanation for runner's amenorrhea.

Consequently, attention must also be focused on the pituitary sensitivity to luteinizing hormone–releasing hormone and the factors that affect it, particularly the effect of gonadal steroids. Women with secondary amenorrhea have been reported to be hypersensitive to the negative feedback effects of estradiol.[14] Since estrogen can increase the amount of pituitary luteinizing hormone in the "storage" pool,[15] which can be released after large doses of luteinizing hormone–releasing hormone such as that used in the current study, such estrogen hypersensitivity might be expected to produce enhanced gonadotropin responses to luteinizing hormone–releasing hormone, such as we have reported. Moreover, since estrogen also may reduce luteinizing hormone secretion from the acutely releasable pituitary pool,[15] from which might come the luteinizing hormone released from the pituitary by the small endogenous luteinizing hormone–releasing hormone pulses seen in amenorrheic runners, estrogen hypersensitivity might result in reduced gonadotropin output and amenorrhea. Thus it is possible that runner's amenorrhea develops as a result of the combination of reduced luteinizing hormone–releasing hormone secretion and/or increased pituitary sensitivity to estrogens. Alternatively, development of exaggerated gonadotropin responses to luteinizing hormone–releasing hormone may simply be a consequence of deficient luteinizing hormone–releasing hormone output per se. For example, when pulsatile luteinizing hormone–releasing hormone support is withdrawn from patients with hypogonadotropic hypogonadism, subsequent gonadotropin responses to bolus luteinizing hormone–releasing hormone can be supranormal.[16] Our data provide no specific information to confirm or refute these theories, which must remain speculative in the absence of confirmatory data. However, our study does define specific abnormalities in amenorrheic runners, confirming the probable hypothalamic-pituitary origin of exercise-induced amenorrhea and suggesting that it may be different from that seen with weight loss.

We would like to thank Mrs. Estelle C. Coleman for expert editorial assistance.

REFERENCES

1. Bullen BA, Skrinar GS, Beitins IZ, Van Mering G, Turnbull BA, McArthur JW. Induction of menstrual disorders by strenuous exercise in untrained women. N Engl J Med 1985;312:1349-53.
2. Chang FE, Dodds WG, Sullivan M, Kim MH, Malarkey WB. The acute effects of exercise on prolactin and growth hormone secretion: comparison between sedentary women and women runners with normal and abnormal menstrual cycles. J Clin Endocrinol Metab 1986; 62:551-6.
3. Boyden TW, Pamenter RW, Grosso D, Stanforth P, Rotkis T, Willmore JH. Prolactin responses, menstrual cycles, and body composition of women runners. J Clin Endocrinol Metab 1982;54:711-4.
4. Loucks AB, Horvath SM. Exercise-induced stress responses of amenorrheic and eumenorrheic runners. J Clin Endocrinol Metab 1984;59:1109-20.
5. Ronkainen H. Depressed follicle-stimulating hormone, luteinizing hormone, and prolactin responses to the luteinizing hormone–releasing hormone, thyrotropin-releasing hormone, and metoclopramide test in endurance runners in the hard-training season. Fertil Steril 1985; 44:755-9.
6. Veldhuis JD, Evans WS, Demers LM, Thorner MO, Wakat D, Rogol AD. Altered neuroendocrine regulation of gonadotropin secretion in women distance runners. J Clin Endocrinol Metab 1985;61:557-63.
7. McArthur JW, Bullen BA, Beitins IZ, Pangano M, Badger TM, Klibanski A. Hypothalamic amenorrhea in runners of normal body composition. Endocr Res 1980;7:13-25.
8. Boyden TW, Pamenter RW, Standforth PR, Rotkis TC, Wilmore JH. Impaired gonadotropin responses to gonadotropin-releasing hormone stimulation in endurance-trained women. Fertil Steril 1984;41:359-63.

9. Yen SSC, Rebar R, Vandenberg G, Judd H. Hypothalamic amenorrhea and hypogonadotropinism: responses to synthetic LRF. J Clin Endocrinol Metab 1973;36:811-6.
10. Anapliotou M, Panitsa-Faflia C, Pitoulis S, Lipraki M, Batrinos ML. Study of the pituitary gonadotropin pools and the positive feedback mechanism of LH release in psychogenic and postpartum amenorrhea. Acta Endocrinol 1982;248(suppl):5-6.
11. Yaginuma T. Progress and therapy of stress amenorrhea. Fertil Steril 1979;32:36-9.
12. Cumming DC, Vickovic MM, Wall SR, Fluker MR, Belcastro AN. The effect of acute exercise on pulsatile release of luteinizing hormone in women runners. Am J Obstet Gynecol 1985;153:482-5.
13. Cumming DC, Vickovic MM, Wall SR, Fluker MR. Defects in pulsatile LH release in normally menstruating runners. J Clin Endocrinol Metab 1985;60:810-2.
14. Santen RJ, Friend JN, Trojanowski D, Davis B, Samojlik E, Barden CW. Prolonged negative feedback suppression after estradiol administration: proposed mechanism of eugonadal secondary amenorrhea. J Clin Endocrinol Metab 1978;47:1220-9.
15. Yen SSC, Lein A. The apparent paradox of the negative and positive feedback control system on gonadotropin secretion. Am J Obstet Gynecol 1976;126:942-54.
16. Valk TW, Corley KP, Kelch RP, Marshall JC. Hypogonadotropic hypogonadism: hormonal response to low dose pulsatile administration of gonadotropin-releasing hormone. J Clin Endocrinol Metab 1980;51:730-8.

The effect of low-dose oral contraceptives on cardiorespiratory function, coagulation, and lipids in exercising young women: A preliminary report

Morris Notelovitz, M.D., Ph.D., Chris Zauner, Ph.D., Lynda McKenzie, R.N., M.Ed., Yvonne Suggs, R.N., Carol Fields, M.P.E., and Craig Kitchens, M.D.
Gainesville and Miami, Florida

A study was undertaken to determine whether low-dose oral contraceptive usage would negate the beneficial effect of exercise on cardiorespiratory fitness, lipid and lipoprotein levels, and coagulation. Twelve exercising women were randomly allocated to groups of either oral contraceptive users or non–oral contraceptive users. When compared with results in the control group, maximal oxygen uptake ($ml/kg^{1} \cdot min^{1}$) decreased significantly in the oral contraceptive users during the 6-month period of observation. This was associated with an 8% decrease in both the oxygen uptake (2.34 to 2.17 L/min) and the oxygen pulse (12.1 ± 3.2 to 11.2 ± 2.2 ml/beat). The serum cholesterol, triglycerides, high-density lipoprotein/cholesterol, and high-density lipoprotein subfractions 2_a and 2_b levels were not altered. A significant increase in plasminogen activity was found in the oral contraceptive users: values increased from a coherent time average of 3.8 ± 0.5 U/ml at baseline to 5.7 ± 0.7 U/ml at 6 months; values returned to baseline levels 1 month after stopping the oral contraceptives (coherent time average of 3.9 ± 0.6 U/ml; $p < 0.0001$). No other significant changes were noted in the coagulation and anticoagulation factors studied. Low-dose oral contraceptive usage is associated with a decrease in functional aerobic capacity, but it does not impinge on the hemostatic mechanism or lipid-lipoprotein metabolism. (Am J Obstet Gynecol 1987;156:591-8.)

Key words: Oral contraceptives, coagulation, fibrinolysis, lipids, lipoproteins, exercise, cardiorespiratory function

Exercise is said to be "cardioprotective." Although this effect may not translate into longevity for all who exercise, there is compelling evidence that the exercise level is indeed inversely related to cardiovascular and respiratory morbidity and mortality.[1] Premenopausal women appear to have a relative immunity to cardiovascular disease. The use of oral contraceptives, by their potential adverse effect on the lipid and lipoprotein profile and on the coagulation system, could reduce this physiologic advantage and negate positive changes induced by exercise. Since much of the earlier information in the literature is based on "high-dose" oral contraceptive usage in nonexercising populations, a

From The Center for Climacteric Studies, Inc., the Department of Internal Medicine, University of Florida, and the Sports Medicine Institute, Mt. Sinai Medical Center.
This study was funded in part by a grant from Mead Johnson Laboratories.
Received for publication August 5, 1986; accepted September 15, 1986.
Reprint requests: Morris Notelovitz, M.D., Ph.D., The Center for Climacteric Studies, Inc., 222 S.W. 36th Terrace, Suite C, Gainesville, FL 32607.

Table I. Characteristics of patients studied

Oral contraceptive use	*Age (yr)*	*Height (cm)*	*Weight (kg)*	
			Baseline	*Conclusion*
Nonusers (group 1)	24.3 ± 3.0	165.2 ± 7.1	57.3 ± 8.0	57.5 ± 8.2
Users (group 2)	24.5 ± 3.2	166.7 ± 3.0	57.5 ± 6.5	59.3 ± 6.3

study was initiated to evaluate the effect of low-dose oral contraception on cardiorespiratory function, lipids and lipoprotein metabolism, and coagulation in exercising young women.

Material and methods

Subjects were recruited from students attending the University of Florida who met the following criteria: they were required to be between 20 to 30 years of age and not currently using oral contraceptives. Their exercise program involved training at least three times per week; runners needed to cover at least 15 miles a week and swimmers 6500 yards a week.

Twelve young women who met the above criteria participated in and completed the study. They had all been in training for more than 1 year. Subjects were randomly assigned into a birth control (n = 6) or a diaphragm/intrauterine device group (n = 6). The oral contraceptive used (Ovcon 35, Mead Johnson, Evansville, Indiana) contained 35 μg of ethinyl estradiol and 0.4 mg of norethindrone. Diaphragm and/or intrauterine devices were fitted for women randomized into the control group. The nonusers of steroid contraceptives (group 1) were closely matched with oral contraceptive users (group 2) (Table I). They were all in good health without evidence of heart or lung disease and without contraindications for oral contraceptive use.

The cardiorespiratory fitness tests were performed at The Center for Physical and Motor Fitness, University of Florida, and the metabolic studies at The Center for Climacteric Studies, Inc., Gainesville. After signing informed consent, each subject had a detailed physical and gynecologic examination. Blood pressure was measured in the sitting position after a 10-minute rest period. The systolic pressure was recorded with the appearance of the first sound, and the diastolic blood pressure with the disappearance of the second sound (phase V Korotkoff). The graded exercise test took place between 6 and 8 AM after a 12-hour complete fast and at least 8 hours of sleep the previous night. The treadmill protocol of Balke et al.,[2] modified for women, was used. The treadmill speed was a constant 3.0 mph and the work task began at a 0% treadmill grade. Elevations in the grade were made at the end of each minute of walking. The first increase was 2%, with subsequent elevations of 1%. After 5 minutes of walking, the subject was performing at 3.0 mph at 5% treadmill grade. This work task was submaximal for all subjects. During the submaximal period, they were evaluated for expired volume in 1 minute, frequency of breathing, tidal volume, oxygen uptake, heart rate, oxygen pulse, and ventilatory equivalent. An automated system (Metabolic Measurements Cart, Beckman Instruments, Schiller Park, Illinois), which performs infrared analysis and polarographic measurement of carbon dioxide and oxygen, respectively, was used to assess all parameters, except for heart rate, which was monitored by palpation.

After measures at submaximal exercise, each subject was brought to maximal physical effort by continuation of the modified protocol of Balke et al.[2] until complete fatigue was proclaimed. The oxygen uptake was measured at this point (maximal oxygen uptake). Two submaximal and two maximal tests were performed on separate days. The assessment reflective of the highest oxygen uptake measure provided the data for analysis. Test days were separated by no fewer than 3 days and no more than 7 days. Each subject was tested before receiving contraception (baseline), and at the conclusion of their respective "treatment."

Blood samples were obtained at the start of the study, at 3 and 6 months of oral contraceptive usage and after oral contraceptives had been suspended for 1 month—the 7-month interval. The sampling was performed at The Center for Climacteric Studies, Inc., between 8 and 9 AM after an overnight fast of 12 hours. The blood for the coagulation profile was collected in tubes containing 3.8% sodium citrate and centrifuged immediately at 4500 rpm for 5 to 10 minutes. Serum was separated and kept frozen at −70° C until analysis. All samples were drawn in the second half of the menstrual cycle.

Tests representative of both coagulation and anticoagulation were performed. The prothrombin time, activated partial thromboplastin time, and the thrombin time were measured by the fibrometer method. Clottable fibrinogen was estimated by the Clauss method.[3] Fibrinogen antigen, plasminogen antigen, α_1-antitrypsin antigen, α_2-macroglobulin antigen, and antithrombin III antigen were determined by radial immunodiffusion with use of plates purchased from Calbiochem-Behring, La Jolla, California (m-Partigen). Plasminogen activity, antithrombin III activity, and α_2-antiplasmin activity were determined by fluorome-

Table II. The effect of a low-dose oral contraceptive on cardiorespiratory function in exercising young women

Test	Group	Submaximal		Maximal	
		Baseline	Conclusion	Baseline	Conclusion
Oxygen uptake ($ml/kg^{-1} \cdot min^{-1}$)	1	15.0 ± 1.0	15.2 ± 1.3	42.6 ± 2.8	45.9 ± 5.8
	2	14.9 ± 0.8	15.9 ± 1.6	41.2 ± 11.8	38.4 ± 9.8
Heart rate (bpm)	1	111.7 ± 17.4	110.0 ± 18.5	195.8 ± 10.1	195.0 ± 5.0
	2	118.8 ± 19.6	132.0 ± 15.3	208.3 ± 26.4	193.2 ± 8.9
Oxygen pulse (ml/beat)	1	7.8 ± 1.3	8.0 ± 1.0	12.3 ± 2.3	13.4 ± 2.2
	2	7.3 ± 1.9	6.6 ± 1.1	12.1 ± 3.2	11.2 ± 2.2
Expired volume (L/min)	1	19.3 ± 2.1	19.9 ± 2.1	77.0 ± 18.7	82.6 ± 15.1
	2	21.7 ± 3.8	22.6 ± 4.6	85.7 ± 23.1	76.7 ± 16.2
Tidal volume	1	0.9 ± 0.2	0.9 ± 0.2	1.9 ± 0.3	1.9 ± 0.3
	2	1.1 ± 0.2	1.0 ± 0.1	1.8 ± 0.3	1.7 ± 0.3

Group 1, Nonusers of oral contraceptives. Group 2, Oral contraceptive users.

tric assays (Protopath, American Dade, Miami, Florida). The variation for Protopath for antithrombin III is 7% and for plasminogen, 5%. All tests were performed by a technician who did not know the identify of the group assignments.

Aliquots of the blood samples were also taken for analysis of serum cholesterol, triglyceride, total high-density lipoprotein, high-density lipoprotein/cholesterol, and high-density lipoprotein subfractions 2_a and 2_b. Serum was obtained by centrifugation for the lipid studies, and the sample divided into two portions. One portion was stored at −60° C until analysis for total cholesterol, high-density lipoprotein/cholesterol, and total triglycerides. The density of the other portion was adjusted to 1.21 gm/cc, with use of sodium bromide. One hundred fifty microliters of the adjusted solution was placed in a Beckman Airfuge centrifuge tube and spun at top speed for 5 hours. After centrifugation 6 μl was removed from the top of the tube and placed directly on a 4% to 30% gradient polyacrylamide gel (Pharmacia Fine Chemical Co., Piscataway, New Jersey). Electrophoresis was carried out for 18 hours at 125 V constant voltage. After electrophoresis the gels were removed from the cassettes and stained for 20 minutes with a solution of 1% amido black in 25% isopropanol, and 10% acetic acid. After electrophoretic destaining the gels were sliced vertically and scanned at 620 nm with use of a Beckman gel scanning attachment coupled to a Model 25 UV-VIS spectrophotometer. Concentrations of high-density lipoprotein subfractions 2_a and 2_b were determined from the recorded scans with use of a Zeiss MOP3 digital pad coupled to a microcomputer. The area under each region of the scan was taken to be equivalent to the protein concentration present. Standard curves were prepared with use of known amounts of separate purified high-density lipoprotein subclasses. Accuracy of determination of separate subclasses was such that <10% variation existed between duplicate samples.

Total serum cholesterol and high-density lipoprotein/cholesterol were determined with use of the cholesterol-oxidase method (Fisher Scientific Products). Non–high-density lipoproteins were precipitated with use of phosphotungstic acid and magnesium. After centrifugation the cholesterol content of the clear supernatant produced was assumed to represent high-density lipoprotein/cholesterol. Total serum triglyceride concentrations were determined with use of a nicotinamide-adenine dinucleotide–linked enzymatic assay (American Monitor Corporation). All methods were standardized with use of the standard solutions supplied by the manufacturers of test kits.

A final physical examination was performed 1 month after completion of their treatment schedule.

Statistical analysis. The data were analyzed with use of repeated measures analysis of variance to test for overall differences between the observation times (baseline, 1, 6, and 7 months) for each parameter measured. If a significant time effect was noted, Tukey's Studentized range test, also known as the HSD (honestly significant difference) test, was used. If the HSD test failed to show any significant differences despite a time effect, Duncan's multiple range test was used to find which time points were significantly different.[4, 5]

Results

The maximal oxygen uptake is the amount of oxygen taken up by the entire body during maximal physical effort. It reflects the status of the entire oxygen delivery system—heart, lungs, blood vessels, blood pool, and the ability of skeletal and myocardial muscle to extract oxygen from the blood. The maximal oxygen uptake in the oral contraceptive users decreased from 41.2 ± 11.8 $ml/kg^2 \cdot min^1$ to 38.4 $ml/kg^1 \cdot min^1$ (a difference of 7%). The maximal oxygen uptake of the group 1 subjects increased their values from 42.6 ± 2.8 to 45.9 ± 5.8 $ml/kg^1 \cdot min^1$ for the baseline and concluding assessments, respectively ($p < 0.03$). The submax-

Table III. The effect of a low-dose oral contraceptive on blood pressure in exercising young women

Blood pressure	*Group*	*Baseline*	*No. of months*		
			3	*6*	*7*
Systolic (mm Hg)	1	105.3 ± 12.3	105.3 ± 5.0	107.0 ± 4.1	108.2 ± 12.4
	2	106.3 ± 5.4	108.7 ± 3.7	111.2 ± 11.6	112.0 ± 11.1
Diastolic (mm Hg)	1	70.7 ± 9.3	66.0 ± 3.6	64.7 ± 3.3	74.4 ± 10.5
	2	66.3 ± 4.8	67.3 ± 4.7	64.0 ± 6.7	74.0 ± 6.8

Group 1, Nonusers of oral contraceptives. Group 2, Oral contraceptive users.

Table IV. The effect of a low-dose oral contraceptive on the coagulation system in exercising women

Test	*Group*	*Baseline*	*No. of months*			*p*
			3	*6*	*7*	
Prothrombin time (normal: 9.5-12.05)	1	12.6 ± 0.6	12.5 ± 0.8	12.2 ± 0.3	12.4 ± 0.9	NS
	2	12.4 ± 0.9	11.2 ± 1.1	11.5 ± 0.3	12.3 ± 0.7	NS
Activated partial thromboplastin time (normal: 35s)	1	29.7 ± 3.1	26.5 ± 2.1	25.9 ± 2.6	25.6 ± 2.3	NS
	2	28.8 ± 5.5	27.5 ± 3.7	25.7 ± 3.7	27.7 ± 5.0	NS
Thrombin time (normal: 10-25s)	1	14.6 ± 1.5	12.5 ± 2.1	15.0 ± 2.7	14.5 ± 1.8	NS
	2	14.5 ± 2.0	16.6 ± 1.9	16.5 ± 2.9	13.8 ± 1.7	0.007
Fibinogen antigen (normal: 200-450 mg/dl)	1	187.3 ± 45.5	169.8 ± 36.5	168.8 ± 50.2	168.2 ± 42.9	NS
	2	177.0 ± 44.0	190.5 ± 41.4	198.3 ± 49.1	200.2 ± 54.9	NS

Group 1, Nonusers of oral contraceptives. Group 2, Oral contraceptive users.

imal oxygen uptake was constant at both test intervals in both groups, as were most of the other cardiorespiratory parameters measured. The only other changes of significance were the oxygen pulse values, that is, the volume of oxygen consumed per heart beat. The non–oral contraceptive users increased their values by 9% (12.3 ± 2.3 to 13.4 ± 2.2 ml/beat), while the treated group's values decreased from 12.1 ± 3.2 to 11.2 ± 2.2 ml/beat—a difference of 8% ($p < 0.02$) (Table II). The systolic and diastolic blood pressures were unaltered in both groups throughout the 7-month study period (Table III).

Two changes among the oral contraceptive users were noted among the dynamic tests of coagulation. The prothrombin time decreased during treatment and returned to baseline values 1 month after cessation of oral contraceptive usage ($p < 0.07$) (Table IV); the thrombin time increased significantly ($p < 0.007$) from a baseline value of 14.5 ± 2.0 S to 16.6 ± 2.0 and 16.5 ± 2.9 S at 3 and 6 months, respectively. The values returned to basal levels at 7 months (13.8 ± 1.7 S). All of these changes were within the range of normal for our laboratory.

The results of the inhibitory component of the anticoagulation system (Table V) were considered separately from those involved with fibrinolysis (Table VI). The most significant finding was that there were no changes in either the antithrombin III antigen or antithrombin III activity. A statistically significant ($p < 0.0001$), treatment-related (group 2) increase in one of the minor anticoagulant enzymes, α_1-antitrypsin antigen, was recorded, but the values remained within physiologic levels.

The values for plasminogen activity and antigen were unaltered in the non–oral contraceptive users (group 1) but were significantly increased in the oral contraceptive group. The baseline plasminogen activity values in group 2 subjects (coherent time average of 3.8 ± 0.5 U/ml) increased to 5.2 ± 0.9 U/ml at 3 months and to 5.7 ± 0.7 U/ml at 6 months. After 1 month without therapy the values had decreased to the baseline levels again ($p < 0.0001$). The 3- and 6-month values exceeded the range of normal for our laboratory. The plasminogen antigen results were similarly increased ($p < 0.0001$), but the actual values were within the laboratory's normal range (Table VI). Antiplasmin activity in the oral contraceptive users decreased while receiving treatment: baseline mean values of 82.0% ± 22.0% fell to 64.3% ± 17.0% and 71.9% ± 13.0% at 3 and 6 months of treatment, with a return to normal at 86.5% ± 6.8% after withdrawal from treatment for 1 month. This change was not, however, statistically significant ($p < 0.06$). There was no change in α_2-antiplasmin values of the nonusers of oral contraceptives.

As reflected in Table VII, none of the lipid (cholesterol and triglyceride) and lipoprotein (total high-density lipoprotein; high-density lipoprotein/cholesterol; high-density lipoprotein subfraction 2_a and 2_b) parameters analyzed were altered during the course of the study. This was true for both the oral contraceptive users (group 2) and the nonusers of oral contraceptives (group 1).

Table V. The effect of a low-dose oral contraceptive on anticoagulation in exercising women

Test	Group	Baseline	No. of months			p
			3	6	7	
Antithrombin III activity (normal: 80-120%)	1	106.7 ± 15.6	105.4 ± 9.6	111.2 ± 8.0	111.7 ± 7.9	NS
	2	104.9 ± 7.9	101.4 ± 6.7	102.5 ± 8.1	106.3 ± 3.6	NS
Antithronbin III antigen (normal: 22-39 mg/dl)	1	26.7 ± 2.5	28.0 ± 3.3	26.3 ± 2.6	28.3 ± 4.3	NS
	2	21.4 ± 4.3	20.1 ± 6.0	21.1 ± 4.5	21.5 ± 4.4	NS
α_1-Antitrypsin antigen (normal: 200-400 mg/dl)	1	165.8 ± 32.3	153.3 ± 25.0	143.3 ± 29.4	161.7 ± 26.2	NS
	2	150.8 ± 23.8	260.0 ± 27.4	271.7 ± 32.9	203.3 ± 63.5	0.0001
α_2-Macroglobulin antigen (normal: 175/450 mg/dl)	1	9.0 ± 68.0	315.0 ± 80.1	295.3 ± 63.3	314.0 ± 74.5	NS
	2	275.3 ± 63.9	284.3 ± 58.0	292.7 ± 55.0	268.7 ± 56.9	NS

Group 1, Nonusers of oral contraceptives. Group 2, Oral contraceptive users.

Table VI. The effect of a low-dose oral contraceptive on plasminogen and α_2-antiplasmin in exercising women

Test	Group	Baseline	No. of months			p
			3	6	7	
Plasminogen activity (normal: 2.4-3.8 CTA U/ml)	1	3.4 ± 0.4	3.5 ± 0.5	3.7 ± 0.5	3.5 ± 0.4	NS
	2	3.8 ± 0.5	5.2 ± 0.9	5.7 ± 0.7	3.9 ± 0.6	0.0001
Plasminogen antigen (normal: 10-20 mg/100 ml)	1	10.2 ± 2.3	10.8 ± 2.3	11.2 ± 2.8	10.3 ± 3.1	NS
	2	10.5 ± 1.5	14.4 ± 1.8	16.1 ± 2.7	11.5 ± 1.9	0.0001
α_2-Antiplasmin activity (normal: 80%-120%)	1	95.0 ± 13.8	91.4 ± 15.1	94.2 ± 10.7	92.6 ± 18.0	NS
	2	82.0 ± 22.0	64.3 ± 17.0	71.9 ± 13.0	86.5 ± 6.8	NS

CTA, Coherent time average. Group 1, Nonusers of oral contraceptives. Group 2, Oral contraceptive users.

Comment

There is a remarkable interest among women to participate in various types of exercise conditioning programs in order to enhance their physical fitness and to maintain their health. Earlier studies have shown that consistent and meaningful exercise is associated with a lower incidence of cardiovascular-related disease[1] and that this can be measured objectively by various parameters, for example, a lowering of and a maintenance of blood pressure in regularly exercising men and women[6]; an improvement in fibrinolytic activity[7]; the well-documented enhancement of the cardioprotective lipoprotein high-density lipoprotein/cholesterol[8] and increases in functional aerobic capacity determined by graded exercise testing.[9]

Another significant health care change for women in recent times has been the use of oral contraceptives. Apart from the efficacy of oral contraceptives in family planning, their long-term use has resulted in many noncontraceptive advantages: a decrease in benign breast disease, ovarian tumors, anemia, and pelvic inflammatory disease, to name a few.[10] However, earlier studies showed that oral contraceptives may have an adverse effect on the cardiovascular system as evidenced by an increased incidence of thrombogenic cardiovascular events, changes in the coagulation/anticoagulation system,[11] a lowering of high-density lipoprotein/lipoproteins,[12] and the development of hypertension.[13] Much of the data regarding these side effects were from studies in women on relatively high-dose estrogen/progestogen preparations, but their physical activity level was rarely commented on. It can be safely assumed that the majority were not involved in formal exercise programs.

The present study has illustrated that the potential benefits of exercise are affected by low-dose steroid contraceptives. Our subjects (according to accepted normative data) were physically fit, since their mean maximal oxygen uptake values, 42.6 ± 2.8 (group 1) and 41.2 ± 11.8 (group 2) $ml/kg^{-1} \cdot min$, were within the range of normal for women of their age: 38 to 48 $ml/kg^{-1} \cdot min^{-1}$. All of the subjects maintained that they continued with their regular exercise program, yet the women receiving oral contraceptives as a group had a significant decrease in maximal oxygen uptake after 6 months of oral contraceptive use—from 41.2 ± 11.8 to 38.4 ± 9.8 $ml/kg^{-1} \cdot min^{-1}$ ($p < 0.03$). Despite an increase in the subjects' weight, which could have accounted in part for this result (from a baseline value of 57.5 ± 6.5 kg to 59.3 ± 6.3 kg), the oxygen uptake at the respective intervals decreased from 2.4 ± 0.67 L/min and 2.2 ± 0.5 L/min (8%), while the oxygen pulse showed a similar decrease (8%) from 12.1 ± 3.2 to 11.2 ± 2.2 ml/beat. The reduction in maximal oxygen uptake did not affect the subjects' regular exercise routines, and the submaximal oxygen uptake, which is

Table VII. The effect of a low-dose oral contraceptive on the lipid and lipoprotein profile (mg/dl) of exercising women

Test	Group	Baseline	No. of months			p
			3	6	7	
Cholesterol	1	146.5 ± 14.4	158.8 ± 26.5	165.2 ± 20.7	140.5 ± 26.4	NS
	2	158.0 ± 29.3	154.0 ± 23.0	151.7 ± 22.3	149.3 ± 22.5	NS
Triglyceride	1	48.7 ± 18.3	65.8 ± 28.3	37.8 ± 9.0	57.3 ± 10.8	NS
	2	38.8 ± 19.6	49.3 ± 17.0	41.5 ± 22.5	38.2 ± 21.4	NS
High-density lipoprotein/cholesterol	1	52.8 ± 4.9	51.3 ± 12.9	49.3 ± 12.2	48.2 ± 9.4	NS
	2	56.3 ± 15.4	52.7 ± 13.4	47.5 ± 14.1	49.2 ± 15.7	NS
High-density lipoprotein/total (mg/dl)	1	288.5 ± 31.2	280.0 ± 72.0	268.2 ± 66.0	260.0 ± 49.8	NS
	2	300.0 ± 76.7	280.0 ± 70.0	256.0 ± 68.2	268.1 ± 104.9	NS
High-density lipoprotein subfraction$_{2a}$ (mg/dl)	1	60.7 ± 25.6	50.5 ± 15.0	50.8 ± 20.9	51.5 ± 12.0	NS
	2	48.5 ± 7.9	32.5 ± 11.7	32.8 ± 9.8	36.2 ± 11.3	NS
High-density lipoprotein subfraction$_{2b}$ (mg/dl)	1	117.0 ± 19.0	94.3 ± 43.4	112.2 ± 42.7	111.3 ± 45.7	NS
	2	135.0 ± 69.0	112.8 ± 52.6	91.7 ± 64.2	84.5 ± 32.3	NS

Group 1, Nonusers of oral contraceptives. Group 2, Oral contraceptive users.

reflective of the energy demands of everyday activity, was unaffected.

Montes et al.[14] also found (as we did) that low-dose oral contraceptives did not have an adverse effect on the ventilatory response to treadmill exercise; in fact, they reported that the oral contraceptive had a moderate ventilatory stimulating effect (not found in this study) and attributed this to the progestin component. Their subjects were all untrained but were also studied for 6 months.

Systolic and diastolic blood pressures remained at normal levels throughout the study. Although the progestogen component of oral contraceptives has been related to elevations in blood pressure, this phenomenon is much less frequent in the lower-dose oral contraceptives.[13] Exercise also has a stabilizing effect on blood pressure,[6] and it has been shown that men and women who are highly fit are less likely to develop hypertension later in life. Although the duration of observation was short and the number of subjects studied was small, low-dose oral contraceptive use did not have a negative impact on the blood pressure of these physically fit young women.

Exercise has an anticoagulant effect and has even been shown to act synergistically with some oral contraceptives to increase fibrinolytic activity, as measured by plasminogen activator, over and above that induced by the oral contraceptive itself.[15, 16] Hedlin et al.[15, 16] in a cross-sectional study that compared 12 women on a medium-dose oral contraceptive (0.05 mg of ethinyl estradiol; 1 mg of ethynodiol diacetate) with 13 matched controls showed that exercise significantly increases both antithrombin III activity and plasminogen activator levels. Their study was based on the influence of acute exercise. In our study involving physically fit women at rest, an increase in plasminogen activity was noted after 3 and 6 months of treatment with the low-dose oral contraceptives. The levels reached, a coherent time average of 5.2 ± 0.9 and 5.7 ± 0.7 U/ml, were well in excess of the reference range for our laboratory (2.4 to 3.8 U/ml) (Table III). The values returned to basal levels 1 month after cessation of oral contraceptive use (3.9 ± 0.6 U/ml). Plasminogen activity levels were unaffected throughout the study in group 1 subjects.

Antiplasmin (α-antiplasmin inhibitor) is a protease that inhibits 35% of the plasmin generated from plasminogen and thus has a most important biologic role in homeostasis. A decrease in this fraction liberates plasmin activity and has been known to cause a dangerous bleeding diathesis. Although the decreased levels noted in the present study were not statistically significant ($p < 0.06$), the values at 3 and 6 months of treatment (64.3% ± 17.0% and 71.9% ± 13.0%) were beyond our laboratory's normal range, reflecting a potential for a true biologic effect. Huisveld et al.[17] recorded a significant decrease in α_2-plasmin inhibitor activity in female cyclists using oral contraceptives. Their subjects, however, were studied within 1 minute of a bicycle ergometer test; acute exercise is known to stimulate fibrinolytic activity. Given the stable α_2-antiplasmin levels in group 1, the decreased α_2-antiplasmin levels during oral contraceptive use (group 2) and the return to basal levels at the 7-month test interval appears to be a treatment effect. The mechanism is not known, but a similar event in a slightly older population of sedentary women has been observed previously and has been attributed to the progestin in the oral contraceptive.[18]

Antithrombin III accounts for at least 50% of the anticoagulant activity of blood. As summarized elsewhere,[19] a number of studies have shown a statistically significant decrease in antithrombin III antigen and activity in women receiving combination oral contraceptives. There are a number of reasons for this, one of which is dose-related; the lower-dose oral contra-

ceptives (those containing 30 to 35 μg of ethinyl estradiol) are associated with either no significant change, or a much reduced effect when compared with higher-dose preparations (≥50 μg of ethinyl estradiol). The level at which antithrombin III would allow thrombus formation is thought to be at 50% of its normal activity. The antithrombin III activity levels in both the treated and control groups remained in excess of 100% throughout the duration of the study (Table V). Exercise-induced increases in this parameter were not observed.[16, 17] The less potent thrombin inhibitors, α_1-antitrypsin and α_2-macroglobulin, may also be regarded as "clot preventors," and they too were either unaffected or increased: α_1-antitrypsin antigen increased significantly ($p < 0.0001$) in group 2 women at 3 and 6 months of treatment (Table V).

Prothrombin times and activated partial thromboplastin times represent activity of the extrinsic and the intrinsic pathways of the coagulation cascade, respectively. Oral contraceptive usage resulted in a decrease in prothrombin time ($p = 0.07$) and a significant decrease in thrombin time ($p < 0.007$). This parameter reflects the conversion of fibrinogen to fibrin and was associated with an observed (but nonsignificant) increase in fibrinogen antigen which persisted for a month after treatment had been stopped. These changes are well within the reference range for our laboratory, and it is unlikely that they represent a change that would result in a biologic effect.

Despite the difficulty of interpreting the clinical effect of the hematologic changes induced by oral contraceptives and the fact that there are no tests that can actually predict clot formation,[20] it is still reasonable to conclude that an increase in overall fibrinolytic activity should counteract thrombus formation.[19] Our study did not set out to determine the effect of exercise per se on the hemostatic mechanism. The results show that the use of oral contraceptives had no adverse effect on the dynamic tests of coagulation and actually enhanced certain factors that may have a proanticoagulant effect.

It is well established that the risk of developing coronary heart disease is inversely related to the level of high-density lipoprotein/cholesterol.[21] This fraction is lowered by oral contraceptives in direct proportion to the potency (or dose) of the progestogen[12] but is increased by exercise.[8] Williams et al.[8] have shown that elevated plasma concentrations of high-density lipoprotein/cholesterol occur only when a threshold exercise level of 10 miles per week is run and is maintained for at least 9 months. It is also important to ensure that study participants are actively training when tested, since a 3-day test can reverse exercise-induced elevations of high-density lipoprotein/cholesterol and lowered serum cholesterol values.[22] All of our study subjects met these requirements. By lowering the progestogen component or the ratio of the progestogen to estrogen, one can anticipate a lesser effect on the high-density lipoprotein/cholesterol moiety.[23] When this regimen is combined with a regular exercise program, no change should therefore occur. This was in fact reflected in the results of the present study. Serum cholesterol and triglycerides, total high-density lipoprotein, high-density lipoprotein/cholesterol and high-density lipoprotein subfraction 2_a levels were unaffected for the entire 7-month duration of the study. The high-density lipoprotein subfraction 2_b level in the oral contraceptive users did show a progressive fall, but the change was not statistically significant. The lipid and lipoprotein levels were compatible with those found in athletes and are in agreement with the study of Gray et al.[24] They studied the effect of oral contraceptives on the lipid profile (serum cholesterol, triglyceride, high-density lipoprotein/cholesterol and low-density lipoprotein/cholesterol) of five women runners and compared these results with six runners who were not receiving oral contraceptives. No significant differences in the lipid profile of the two groups were found in this cross-sectional study.

Given the small sample size and a relatively short duration of observation, it would be unwise to project the conclusion of this study to the general population. Nevertheless, we have shown that low-dose oral contraceptives given to physically fit young women did decrease their functional aerobic capacity and could result in a decrease in the maximal performance ability of athletes. There was no adverse effect on the hemostatic mechanism or the lipid-lipoprotein profile.

The statistical analysis was performed by Jane Pendergast, Ph.D., Department of Biostatistics, University of Florida College of Medicine, and the lipid and lipoprotein analysis by Tom Grow, Ph.D., Department of Basic Dental Science, College of Dentistry, University of Florida.

REFERENCES

1. Paffenberger RS, Hyde TT, Wing AL, Steinmetz CH. A natural history of athleticism and cardiovascular health. JAMA 1984;252:491.
2. Balke B, Grillo GP, Konecci EB, et al. Work capacity after blood donation. J Appl Physiol 1954;7:231.
3. Clauss A. Gerinnunsphysiologische Schnellmethode zur Bestigimmung des Fibrinogens. Acta Haematol 1957;17:237.
4. Duncan DB. t-Tests and intervals for comparisons suggested by the data. Biometrics 1975;31:339.
5. Kramer CV. Extension of multiple range tests to group means with unequal numbers of replications. Biometrics 1956;12:307.
6. Blair S, Goodyear NN, Gibbons LW, Cooper KH. Physical fitness and incidence of hypertension in healthy normotensive men and women. JAMA 1984;252:487.
7. Williams S, Logne EE, Lewis JL, et al. Physical conditioning augments the fibrinolytic response to venous occlusion in healthy adults. N Engl J Med 1980;302:987.
8. Williams PT, Wood PD, Haskell WB, Vranizan K. The

effects of running mileage and duration on plasma lipoprotein levels. JAMA 1982;247:2674.
9. Bruce RA. Exercise, functional aerobic capacity and aging—another viewpoint. Med Sci Sports Exerc 1984;16:8.
10. Mishell DR. Noncontraceptive health benefits of oral steroidal contraceptives. Am J Obstet Gynecol 1982; 142:809.
11. Poller L. Oral contraceptives, blood clotting and thrombosis. Br Med Bull 1978;34:151.
12. Bradley DD, Wingerd J, Pettiti DB, et al. Serum high density lipoprotein cholesterol in women using oral contraceptives, estrogens and progestins. N Engl J Med 1978;299:17.
13. Kay CR. Progestogens and arterial disease—evidence from the Royal College of General Practitioner's Study. Am J Obstet Gynecol 1982;142:762.
14. Montes A, Lally D, Hale RW. The effects of oral contraceptives and respiration. Fertil Steril 1983;3:515.
15. Hedlin AM, Milojevic J, Korey A. Hemostatic changes induced by exercise during oral contraceptive use. Can J Physiol Pharmacol 1978;56:316.
16. Hedlin AM, Milojevic J, Korey A. Plasminogen activator levels in plasma and urine during exercise and oral contraceptive use. Thromb Haemost (Stuttgart) 1978;3:743.
17. Huisveld JA, Hospen AJH, Bernink MJE. Oral contraception and fibrinolysis among female cyclists before and after exercise. Appl Physiol 1982;53:330.
18. Notelovitz M, Levenson I, McKenzie L, Lane D, Kitchens C. The effects of low-dose oral contraceptives on coagulation and fibrinolysis in two high-risk populations: smoking and older premenopausal females. Am J Obstet Gynecol 1985;152:995.
19. Notelovitz M. Oral contraception and coagulation. Clin Obstet Gynecol 1985;28:73.
20. Mammen EF. Oral contraceptives and blood coagulation: a critical review. Am J Obstet Gynecol 1982;142:781.
21. Gordon T, Castelli WP, Hjortland MC, et al. High-density lipoprotein as a protective factor against coronary heart disease: the Framingham Study. Am J Obstet Gynecol 1977;62:707.
22. Dressendorfer RH, Wade CE, Hornick C, Timmis CG. High-density lipoprotein-cholesterol in marathon runners during a 20-day road race. JAMA 1982;247:1715.
23. Notelovitz M, Gudat J, McKenzie L. Contraceptives, lipids, and lipoproteins: a preliminary report. Curr Ther Res 1981;30:627.
24. Gray PD, Harding E, Dale E. Effects of oral contraceptives on serum lipid profiles of women runners. Fertil Steril 1983;3:510.

Reassessment of White's classification and Pedersen's prognostically bad signs of diabetic pregnancies in insulin-dependent diabetic pregnancies

Michael P. Diamond, M.D., Sheron L. Salyer, R.N.C., William K. Vaughn, Ph.D., Robert Cotton, M.D., and Frank H. Boehm, M.D.

Nashville, Tennessee

The classification systems developed over 20 years ago by White and Pedersen identified diabetic pregnancies at increased risk for perinatal mortality. To assess whether these same criteria would currently be valid, 199 diabetic pregnancies with deliveries from 1977 to 1983 were reviewed. Perinatal mortality rates for White's Classes B gestational (n = 72), B (n = 27), C (n = 67), and D + F + R (n = 33) were 2.9%, 11.1%, 14.9%, and 21.1%, respectively ($p < 0.05$). White's classes were also predictive of pulmonary morbidity (12.5%, 18.5%, 22.4%, and 42.4%, respectively). The presence of one or more of the prognostically bad signs of pregnancy (n = 76) increased the perinatal mortality rate to 17.1% versus 7.3% among insulin-dependent diabetic pregnancies without prognostically bad signs ($p < 0.05$). The presence of any prognostically bad signs of pregnancy was also predictive of pulmonary morbidity in general (31.6% versus 16.3%, respectively) and hyaline membrane disease in particular (13.2% versus 4.1%, respectively). Thus with use of modern obstetric management and medical care of the pregnant diabetic patient, both White's classification and Pedersen's prognostically bad signs of pregnancy continue to be predictive of perinatal mortality. (Am J Obstet Gynecol 1987;156:599-604.)

Key words: Diabetes, pregnancy, White's classification, reassessment, Pedersen's prognostically bad signs

In the late 1940s White devised a classification of diabetic pregnancies which established more precise predictions of the perinatal mortality rate.[1] This system was based on prepregnancy characteristics of the mother, namely, the duration of diabetes, the age of onset, and the presence or absence of diabetic vascular complications. Subsequently, in the 1960s Pedersen and associates described another system for predicting perinatal mortality in diabetic pregnancies, which complemented White's classification. This second system identified a poorer outcome in those pregnancies in which one of the following four prognostically bad signs of pregnancy developed during pregnancy: diabetic ketocidosis, pregnancy-induced hypertension, pyelonephritis, or maternal neglect.[2, 3]

However, since the introduction of these predictive classification systems, the medical care, obstetric management, and neonatal treatment of the pregnant diabetic patient and her infant changed dramatically.[4-9] Modern advances include the use and application of ultrasound, assessment of fetal well-being by nonstress tests, oxytocin challenge tests, and biophysical profiles, the prediction of fetal lung maturity, and advances in treatment of prematurity and neonatal respiratory distress.[4] Consequently, questions have arisen as to whether the current application of White's classification and Pedersen's prognostically bad signs continues to possess predictive value in the identification of pregnancies at increased risk for perinatal mortality.

This report examines the value of these classification systems in the prediction of perinatal mortality in insulin-dependent diabetic pregnancies. Furthermore, it evaluates whether White's classification or Pedersen's prognostically bad signs are of predictive value in identifying infants with an increased risk of developing the various types of neonatal morbidity to which infants of diabetic pregnancies are predisposed.

Material and methods

The patient population for this report consisted of all insulin-dependent diabetic women who delivered during a 7-year period from January 1, 1977, to December 31, 1983, at Vanderbilt University Medical Center, a tertiary referral hospital. Data collection was accomplished by a review of the medical records of these women and their infants.

From the Division of Biostatistics and the Departments of Obstetrics and Gynecology and Pediatrics, Vanderbilt University Medical Center.

Presented in part at the Twelfth Annual Congress of the International Diabetes Federation, Madrid, Spain, September 23-28, 1985.

Received for publication February 21, 1986; revised July 17, 1986; accepted October 17, 1986.

Reprint requests: Michael P. Diamond, M.D., Department of Obstetrics and Gynecology, Yale University School of Medicine, 333 Cedar St., P. O. Box 3333, New Haven, CT 06511.

Table I. Outcome of insulin-dependent diabetic pregnancies according to White's classification

	White's Class					
	Bg (n = 72)			*B (n = 27)*		
	n	*Mean ± SEM*	%	*n*	*Mean ± SEM*	%
Maternal factors						
Age (yr)		30.4 ± 0.7			30.1 ± 1.2	
Previous stillbirths	10		13.9	4		14.8
Estimated gestational age at delivery (wk)		37.3 ± 0.3			36.3 ± 0.7	
Total hospital days		11.4 ± 0.8			11.9 ± 1.4	
Neonatal factors						
Nursery						
Normal	41		57.7	13		50.0
Neonatal intensive care unit	30		42.2	13		50.0
Apgar score						
1 minute		6.9 ± 0.2			6.2 ± 0.5	
5 minute		8.4 ± 0.1			7.6 ± 0.5	
Weight (gm)		3229 ± 84			2954 ± 181	
Macrosomia	21		29.2	10		37.0
Birth injuries	4		5.6	0		0.0
Respiratory distress (total)	9		12.5	5		18.5
Hyaline membrane disease	4		5.6	1		3.7
Hypoglycemia	9		12.5	10		37.0
Hypocalcemia	1		1.4	3		11.1
Hyperbilirubinemia	18		25.0	3		11.1
Polycythemia	2		2.9	0		0.0
Congenital anomalies	5		6.9	6		22.2
Death*						
Intrauterine fetal death	1		1.4	1 (1)*		3.7
Nursery	1 (1)		1.4	2 (2)		7.4
Total	2 (1)		2.9	3 (3)		11.1
Total hospital days		6.3 ± 0.4			6.3 ± 0.9	

*Numbers in parentheses indicate deaths associated with congenital malformations.

Management of these insulin-dependent diabetic pregnancies has been previously described.[9] Briefly stated, the principle of obstetric care of these women has been an increasing reliance on outpatient management while awaiting development of fetal pulmonary maturity, at which time delivery was initiated. Hospital admissions are now limited to those for obstetric indications or delivery.

The current management plan includes evaluation of glucose homeostasis at the initial presentation for insulin-dependent diabetes or as soon as the need for insulin management of gestational diabetes was determined.[5] This evaluation was performed at home and was composed of 3 days of measuring glucose "day curves." Day curves consisted of blood glucose levels measured in the fasting state and postprandial levels measured 2 hours after breakfast, lunch, and dinner. Target glucose values were <100 mg/dl during fasting and <150 mg/dl after meals. During this introduction period the patients were instructed in the home measurement of day curves by means of Chemstrips (Bio-Dynamics, Indianapolis, Indiana), the composition of an American Diabetes Association diabetic diet of approximately 35 calories per kilogram of ideal body weight, the measurement of urine ketones, the administration of insulin, and the principles of diabetic care. These women subsequently performed a day curve measurement once a week and phoned these results to the nurse clinician who would consult with a physician regarding possible alterations in insulin doses. Insulin administration usually consisted of twice daily injections of a mixture of neutral protamine Hagedorn and regular insulin.

The mainstay of our antepartum fetal monitoring system has been the nonstress test, which is currently performed twice weekly beginning at 32 weeks' gestation and continuing until delivery. (During the first 3 years of the study, nonstress tests were performed weekly.) If fetal well-being could not be established by the nonstress test, evaluation was continued with use of contraction stress tests and biophysical profiles as described previously.[5] Pregnancy was continued until the development of spontaneous term labor or until fetal lung maturity could be established by amniocentesis. After demonstration of pulmonary maturity, delivery was initiated by Pitocin induction or cesarean section depending on the cervical status.

Listing of congenital anomalies and birth injuries was

White's Class						
C (n = 67)			D + F + R (n = 33)			
n	Mean ± SEM	%	n	Mean ± SEM	%	p
	23.6 ± 0.5			22.9 ± 0.8		
5		7.5	4		12.1	NS
	36.3 ± 0.4			35.3 ± 0.7		
	13.0 ± 1.1			14.7 ± 3.0		
20		33.3	8		27.6	
40		66.7	21		72.4	<0.01
	6.9 ± 0.3			6.7 ± 0.4		
	8.0 ± 0.3			8.1 ± 0.4		
	3145 ± 126			2602 ± 160		
28		41.8	3		9.1	<0.01
2		3.0	0		0.0	NS
15		22.4	14		42.4	0.013
5		7.5	5		15.2	NS
14		20.9	7		21.2	NS
2		3.0	2		6.0	NS
20		29.9	7		21.2	NS
5		7.5	3		9.1	NS
9		13.4	6		18.2	NS
7 (3)		10.4	4 (2)		12.1	NS
3 (2)		4.5	3 (2)		9.1	NS
10 (5)		14.9	7 (4)		21.2	<0.03
	7.7 ± 1.1			10.6 ± 2.1		

limited to those identified in the nursery. Diagnosis of neonatal pulmonary morbidity in general and hyaline membrane disease in particular was obtained from the neonatal intensive care unit and based on published criteria.[10] Pulmonary morbidity refers to the occurrence of all types of neonatal pulmonary difficulties including hyaline membrane disease, type II respiratory distress syndrome, pulmonary edema, meconium pneumonitis, and pneumonia. Neonatal morbidity was defined for the purpose of this report as previously described:[9] neonatal hypoglycemia as a glucose level of <40 mg/dl in infants weighing ≥2000 gm and a level of <30 mg/dl in infants weighing <2000 gm; hypocalcemia as a calcium level of <7 mg/dl; hyperbilirubinemia as a bilirubin of ≥12 mg/dl;; polycythemia as a hematocrit of ≥70%; macrosomia as a birth weight greater than the ninetieth percentile at each gestational age.

For the purpose of analysis, White's Class B was subdivided into those women who were not receiving insulin before pregnancy (gestational diabetic patients, Bg) and those receiving insulin before pregnancy (B). During the period of the study an attempt was routinely made to manage cases of gestational diabetes by dietary manipulation alone. Those started on insulin were unable to be managed successfully by diet alone or had markedly elevated fasting blood sugar (usually >120 mg/dl). Classes D, F, and R were grouped together to facilitate statistical analysis. As such, this group represents patients with long-standing diabetes and/or diabetic complications. Statistical analysis was performed by the χ^2 test, Fisher's exact test, and the unpaired t test. All data are expressed as mean ± SEM. Statistical significance was defined as $p < 0.05$.

Results

During the 7 years of this evaluation, 199 women with insulin-dependent diabetes mellitus delivered at Vanderbilt University Medical Center. Grouped by White's classification 99 were Class B, of which 72 were gestational (Bg), and 27 were insulin dependent before pregnancy (B). Sixty-seven were Class C, 20 were Class D, six were Class F, and nine were Class R. Maternal age at the time of delivery decreased in association with advancing White's classification (Table I). In a comparison of the estimated gestational age at the time of delivery among the four groups, the only significant difference was between the Bg and D + F + R groups (37.3 ± 0.3 vs. 35.3 ± 0.7 weeks, respectively; $p < 0.05$). Based on White's classification, there was no

Table II. Outcome of recent insulin-dependent diabetic pregnancies with and without Pedersen's prognostically bad signs of diabetic pregnancies

	With prognostically bad signs			*Without prognostically bad signs*			
	n	*Mean ± SEM*	*%*	*n*	*Mean ± SEM*	*%*	*p*
Maternal factors							
Age (yr)		24.5 ± 0.6			28.1 ± 0.6		<0.0005
Previous stillbirths	8		10.5	15		12.2	NS
Estimated gestational age at delivery (wk)		36.2 ± 0.4			36.7 ± 0.3		NS
Total hospital days		14.3 ± 1.1			11.4 ± 0.6		<0.005
Neonatal factors							
Nursery							
Normal	17		25.8	62		51.7	<0.005
Neonatal intensive care unit	49		74.2	58		48.3	
Apgar score							
1 minute		6.4 ± 0.3			7.0 ± 0.2		NS
5 minute		7.8 ± 0.3			8.3 ± 0.1		NS
Weight (gm)		2970 ± 115			3125 ± 76		NS
Macrosomia	24		31.6	38		30.9	NS
Birth injuries	2		2.6	5		4.1	NS
Respiratory distress (total)	24		31.6	20		16.3	<0.02
Hyaline membrane disease	10		13.2	5		4.1	<0.02
Hypoglycemia	14		18.4	27		22.0	NS
Hypocalcemia	5		6.6	3		2.4	NS
Hyperbilirubinemia	20		26.3	28		2.8	NS
Polycythemia	0		0.0	10		8.1	<0.02
Congenital anomalies	11		14.5	15		12.2	NS
Death*							
Intrauterine fetal death	10 (4)*		13.2	3 (2)		2.4	<0.005
Nursery	3 (2)		3.9	6 (5)		4.9	NS
Total	13 (6)		17.1	9 (7)		7.3	<0.04
Total hospital days		8.4 ± 1.0			6.8 ± 0.5		NS

*Numbers in parentheses indicate deaths associated with congenital malformations.

difference in the rate of previous stillbirths or the length of maternal hospitalization during the prenatal and postpartum periods (Table I).

With regard to neonatal morbidity, there was an increased likelihood of admission to the neonatal intensive care unit with advancing White's classification ($p < 0.03$). The length of infant hospitalization was highest in group D + F + R, followed by group C, and finally by both groups Bg and B. There was no difference in Apgar scores at 1 minute, while at 5 minutes the only significant difference was between groups Bg and B. Compared to the D + F + R group, the mean weights of infants in all of the other groups was higher, although this difference was only significant for groups Bg and C. Macrosomia was significantly less common in group D + F + R than in any of the other groups.

There was a significant increase in the rates of neonatal pulmonary morbidity in association with advancing White's classification ($p = 0.0123$). While a similar trend was seen for the occurrence of hyaline membrane disease, it did not reach statistical significance. The rates of hypoglycemia, hypocalcemia, hyperbilirubinemia, and polycythermia were not different between the groups. The rate of congenital anomalies was lowest in the gestational diabetic patients, but there was no statistical significance. The perinatal death rate increased in association with advancing disease according to White's classification. This increase represented increases in both the rate of intrauterine fetal deaths and nursery deaths, although the trend in neither of those groups individually reached statistical significance. (Of the 22 intrauterine and nursery deaths, 13 were due to congenital anomalies incompatible with life.)

Pedersen's prognostically bad signs were identified in 76 pregnancies. The most frequent was pregnancy-induced hypertension, which was identified in 41. Diabetic ketoacidosis occurred in 21 pregnancies; of these infants, seven died intrauterine and one in the nursery. Mothers were characterized as neglectors in 14 pregnancies. Pyelonephritis only occurred during six pregnancies; however, one fetal death was observed in that group.

Table II examines outcome of insulin-dependent diabetic pregnancies grouped by the presence or absence of one or more prognostically bad signs of pregnancy. Mothers with prognostically bad signs were significantly

younger ($p < 0.0005$) and had a greater length of hospitalization ($p < 0.0005$); however, there was no difference in the rate of previous stillbirths or the gestational age at delivery. With regard to neonatal outcome, infants born to pregnancies with a prognostically bad sign were significantly more likely to be admitted to the neonatal intensive care unit ($p = 0.025$), although their total length of hospitalization was not significantly increased. There was no difference in Apgar scores between the two groups, and there was no difference in the birth weights or birth injuries. Polycythemia occurred in 10 infants, all of whom were in the group without prognostically bad signs. There was no difference in the rates of hypoglycemia, hypocalcemia, hyperbilirubinemia, macrosomia, or congenital anomalies. However, pulmonary morbidity in general was significantly increased in the group with prognostically bad signs ($p = 0.0014$), as was the occurrence of hyaline membrane disease in particular ($p = 0.0182$). Finally, the perinatal death rate was significantly higher in the group with prognostically bad signs ($p = 0.0324$). This represented an increased rate of intrauterine fetal death in that group ($p = 0.0029$), which was primarily composed of intrauterine fetal death in women with diabetic ketoacidosis or in neglectors.

Comment

Recognition of the increased risks to which infants of diabetic mothers are predisposed has been described since before the turn of the century.[11] White's classification and Pedersen's prognostically bad signs each characterized diabetic pregnancies, identifying those infants at risk for perinatal mortality.

In her review of 78 fetal deaths in 439 diabetic pregnancies (18%), White[1] concluded that the worst maternal prognostic factor was the degree of vascular disease. Other factors identified included the age of onset of diabetes and its duration. Regarding stillbirths in the diabetic population, White observed that 68% occurred after 35 weeks' gestation, while congenital anomalies were responsible for 15% of the neonatal deaths.

In their report describing 304 diabetic pregnancies Pedersen and Pederson[2] identified a perinatal mortality rate of 18%. Among pregnancies in which a prognostically bad sign was present, the perinatal mortality rate was 32%, as compared to only 8% in pregnancies without prognostically bad signs. Examining the factors that constitute prognostically bad signs individually, the following perinatal mortality rates were identified: pyelorephritis, 41%; diabetic ketoacidosis, 37%; severe toxemia, 38%; less severe toxemia, 24%; neglectors, 38%. White's classification was also found to be predictive of the perinatal mortality rate in their patient population.

The potential quality of diabetic pregnancies can be markedly improved. In a group of patients maintained during pregnancy under meticulous metabolic control, the complications to which infants of diabetics mothers were predisposed were totally eliminated.[12] Other recent reports have also described substantial reductions in the complications of diabetic pregnancies compared to those early reports, with one exception, the continued occurrence of an increased rate of congenital anomalies. The failure to reduce the rate of anomalies is believed to be due to the failure to establish good metabolic control during the period of embryogenesis,[13] since this often occurs before women realize they are pregnant. Further support for this hypothesis was a report describing a significant reduction in the occurrence of anomalies in diabetic women who underwent intensive metabolic regulation before the time of conception.[14] Thus there should be a good prognosis for the outcome of diabetic pregnancies.

In view of the improvements in medical care, obstetric management, and neonatal treatment that have occurred and are reflected in those recent reports, a question has been raised: do these classification systems still provide predictive value with regard to the outcome of diabetic pregnancies? This report examines the degree of attainment of this potential, as well as prognostic significance of White's classification and Pedersen's prognostically bad signs of pregnancy. Since Vanderbilt University Medical Center is a tertiary care referral center, the pregnancies delivered here represent a wide variety of clinical situations. Among the pregnancies described in this report the situations range from that of women who presented for metabolic regulation before the time of conception to women who presented for the first time at our center in diabetic ketoacidosis and with a fetus with intrauterine death.

Reassessment of our patient population by White's classification demonstrated that this classification continues to be of prognostic significance with regard to perinatal mortality. Similarly, Pedersen's prognostically bad signs were also found to continue to be of prognostic significance in the identification of diabetic pregnancies at risk for perinatal mortality. Of further interest, however, is the observation that the perinatal mortality was associated principally with one of three situations: fetal congenital anomalies, maternal neglectors, and women referred to our center late in their gestations with either maternal or fetal complications. Nevertheless, when our patient population as a whole is considered, each of these classification systems maintains its prognostic significance for the identification of perinatal mortality.

This report also identified an association between each classification system and the occurrence of neonatal pulmonary morbidity in infants. Among women with prognostically bad signs of pregnancy, respiratory distress in infants was twice as common as in those women without prognostically bad signs. Similarly, in

a comparison of these two groups, the incidence of hyaline membrane disease in the infants was three times greater. When pregnancies were grouped by White's classification, pulmonary morbidity in general was significantly correlated with advancing disease according to White's classification. There was also a trend for an increased incidence of hyaline membrane disease in association with advanced disease according to White's classification although this did not reach statistical significance. Although each of these latter relationships may have been related to the gestational age of delivery of the infants, it nevertheless establishes that women with a higher White's classification are at increased risk for having infants with respiratory difficulties.

With regard to other types of neonatal outcome, average birth weight by White's classification was least in the D + F + R group. The infants in the D + F + R group were also significantly less likely to have macrosomia (which is based on both the weight and gestational age at delivery). This may indicate a relative impairment in uteroplacental function in these women with advanced classes of diabetes as compared to groups Bg, B, and C. The explanation for the increased occurrence of polycythemia in infants of pregnancies without prognostically bad signs is unclear. Neither advanced White's classification nor prognostically bad signs were predictive of other types of neonatal morbidity.

In summary, this report has established the continued usefulness of both White's classification and Pedersen's prognostically bad signs of diabetic pregnancies in identifying diabetic pregnancies at risk for the occurrence of perinatal mortality. Each of these also appears to be predictive of the occurrence of neonatal respiratory difficulties.

REFERENCES

1. White P. Pregnancy complicating diabetes. Am J Med 1949;7:609.
2. Pedersen J, Pedersen LM. Prognosis of the outcome of pregnancies in diabetes: a new classification. Acta Endocrinol 1965;50:70.
3. Pedersen J, Pedersen LM, Andersen B. Assessors of fetal perinatal mortality in diabetic pregnancy: analysis of 1332 pregnancies in the Copenhagen series, 1946-1972. Diabetes 1974;23:302.
4. Diamond MP, Shah DM, Hester RA, Vaughn WK, Cotton RB, Boehm FH. Complications of insulin-dependent diabetic pregnancies by preeclampsia and/or chronic hypertension: analysis of outcome. Am J Perinatol 1985; 2:263.
5. Diamond MP, Vaughn WK, Salyer SG, Cotton RB, Fields LM, Boehm FH. Antepartum fetal monitoring in insulin-dependent diabetic pregnancies. Am J Obstet Gynecol 1985;253:528.
6. Kitzmiller JL, Cloherty JP, Younger MD, et al. Diabetic pregnancy and perinatal morbidity. Am J Obstet Gynecol 1978;131:560.
7. Gabbe SG, Lowensohn RI, Wu PYK, Guerra G. Current pattern of neonatal morbidity and mortality in infants or diabetic mothers. Diabetes Care 1978;1:335.
8. Golde SH, Montoro M, Good-Anderson B, et al. The role of nonstress tests, fetal biophysical profile, and contraction stress tests in the outpatient management of insulin-requiring diabetic pregnancies. Am J Obstet Gynecol 1984;148:269.
9. Diamond MP, Vaughn WK, Salyer SL, Cotton RB, Fields LM, Bochm FH. Efficacy of outpatient management of insulin-dependent diabetic pregnancies. J Perinatol 1985;5:2.
10. Cotton RB, Stahlman MT, Kovar T, Catterton W. Medical management of small preterm infants with symptomatic patient ductus arteriosus. J Pediatr 1978;92:467.
11. Duncan JM. On puerperal diabetes. Trans Obstet Soc Lond 1882;24:256.
12. Jovanovic L, Peterson CM, Saxena BB, Dawood MY, Sandek CD. Feasibility of maintaining normal glucose profiles in insulin-dependent pregnant diabetic women. Am J Med 1980;68:105.
13. Miller E, Hare JM, Cloherty JP, et al. Elevated maternal hemoglobin AIC in early pregnancy and major congenital anomalies in infants of diabetic mothers. N Engl J Med 1981;304:1331.
14. Fuhrmann K, Reiber H, Semmler K, Fischer F, Fischer M, Glockner E. Prevention of congenital malformations in infants of insulin-dependent diabetic mothers. Diabetes Care 1983;6:219.

Legal abortion mortality in the United States: 1972 to 1982

Hani K. Atrash, M.D., H. Trent MacKay, M.D., Nancy J. Binkin, M.D., and Carol J. R. Hogue, Ph.D.

Atlanta, Georgia

Between 1972 and 1982, 186 women died as a result of legal abortion in the United States. The overall death rate resulting from legal abortion dropped nearly fivefold, from 4.1 per 100,000 abortions in 1972 to 0.8 in 1982. Women who were older, black, of high parity, and had abortions at a later gestational age were at increased risk of death throughout the 11 years of surveillance. During this period, the death rate decreased for abortions at all stages of gestation; the greatest decrease was with abortions performed during the second trimester. For the entire interval, mortality rates were highest for abortions performed by hysterotomy or hysterectomy and lowest for abortions performed by curettage. Before 1977, the most common causes of abortion-related death were infection, hemorrhage, and general anesthesia complications, respectively. Thereafter, hemorrhage became the most common cause of abortion-related death, followed in number by general anesthesia complications. Our findings suggest that there has been a marked decrease in septic legal abortion deaths, but potentially preventable deaths from general anesthesia and hemorrhage remain an important concern. Use of general anesthetics during first-trimester abortions should be carefully reviewed. (Am J Obstet Gynecol 1987;156:605-12.)

Key words: Abortion, mortality, maternal mortality

In 1982, 11 women died as a result of legal abortion in the United States. This represented a 57% increase over the reported number of deaths in 1981 and a 56% increase in the death-to-case rate. In addition, several changes in the characteristics of women both obtaining and dying of legal abortion and in the causes of legal abortion–related deaths have been observed recently.[1] These observations prompted a review of all abortion-related deaths identified through the Centers for Disease Control's abortion mortality surveillance for the period 1972 to 1982.

In this review, we analyze temporal trends in abortion mortality and study the factors that place a woman at increased risk of dying from legal abortion. We also describe the change in the leading causes of death from legal abortion and the change in each risk factor's contribution to abortion mortality over a period of time.

Methods

Abortion-related deaths are identified by the CDC from a variety of sources including the National Center for Health Statistics, state health departments, reports from abortion facilities and medical personnel, state medical and hospital associations, and state maternal mortality review committees. Each reported death is thoroughly investigated by examination of medical records and coroners' reports and is categorized as legal, illegal, spontaneous, or non–abortion related. Abortion-related deaths are defined as deaths resulting from a direct complication of an abortion, an indirect complication caused by the chain of events initiated by the abortion, or an aggravation of a preexisting condition by the physiologic or psychological effects of the abortion. We defined legal abortion as an abortion performed by a licensed physician or someone acting under the supervision of a licensed physician.

We used data from the annual CDC abortion surveillance to provide denominators for the death-to-case rates.[1] Because all states do not report on each variable, we applied the percentage distribution for those variables on which we had information to the total number of reported abortions.

Because of the small number of abortion-related deaths, particularly in the demographic and medical subcategories, for the purposes of comparison we elected to aggregate the data into two temporal groups: deaths occurring from 1972 to 1976 and those occurring from 1977 to 1982.

To describe the risk of death following various abortion procedures, we used four categories of abortion procedures. Suction curettage, sharp curettage, and dilatation and evacuation procedures performed at ≤12 weeks' gestation were categorized as curettage procedures.[1] The second procedure category, dilatation and evacuation, included procedures in the first category that were done at ≥13 weeks' gestation. We aggregated all instillation procedures into a third group, and hys-

From the Division of Reproductive Health and the Division of Nutrition, Center for Health Promotion and Education, Centers for Disease Control, United States Public Health Service.

Received for publication June 30, 1986; revised August 28, 1986; accepted October 17, 1986.

Reprints not available.

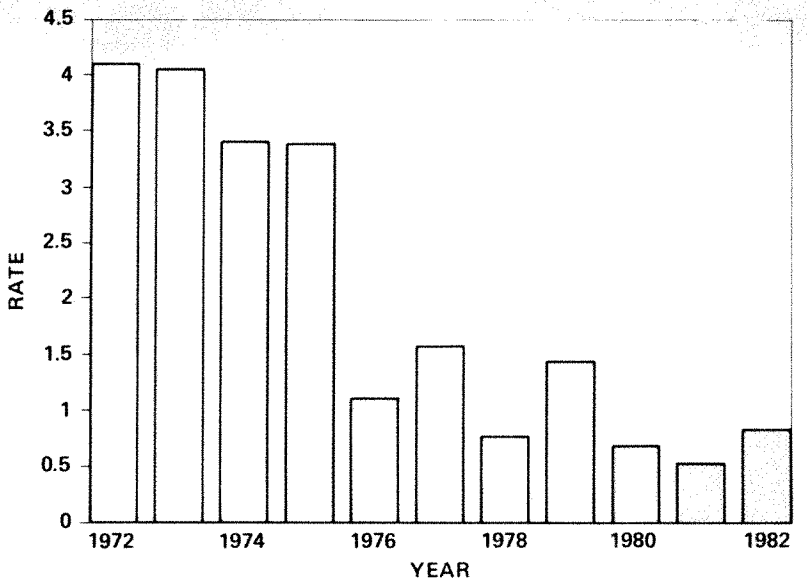


Fig. 1. Legal abortion mortality by year, United States, 1972 to 1982.

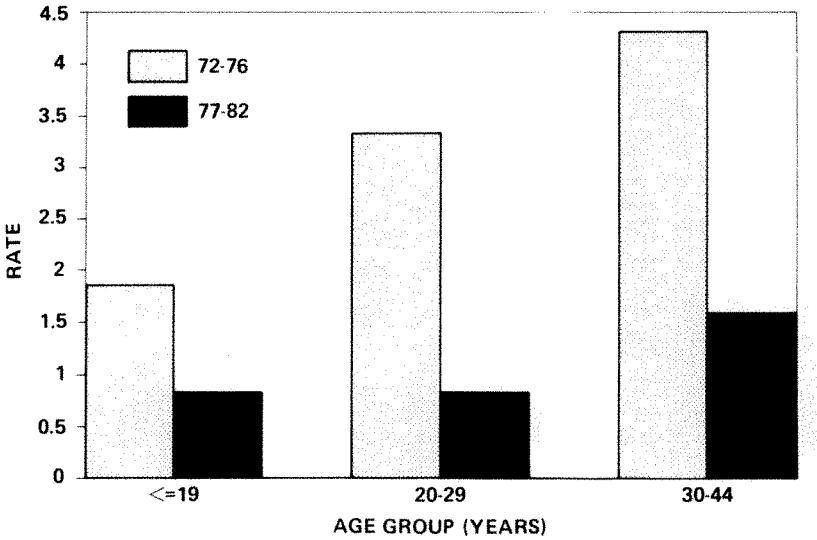


Fig. 2. Legal abortion mortality by age, United States, 1972 to 1976 and 1977 to 1982.

terotomy and hysterectomy procedures were combined to form a fourth group.

The relative risks and confidence intervals were calculated by the Mantel-Haenzel method as described by Rothman and Boice.[2]

Results

One hundred eighty-six legal abortion–related deaths were identified and investigated during 1972 to 1982. The legal abortion mortality rate has decreased steadily since 1972. Overall, the rate dropped 4.9-fold, from 4.1 per 100,000 procedures in 1972 to 0.8 per 100,000 procedures in 1982[1] (Fig. 1). In three instances (1977, 1979, and 1982) the number and rate of abortion deaths were higher than those of the immediately preceding year. For the composite years 1972 to 1976, the abortion mortality rate was 3.0 per 100,000 procedures, whereas for 1977 to 1982, the abortion mortality rate was 1.0 per 100,000 procedures.

Age. The risk of legal abortion–related mortality increased with age (Table I), but the magnitude of the risk decreased over the period of time. Between 1972 and 1976, women aged ≤19 years had a death-to-case rate of 1.9 per 100,000 in comparison with a death-to-case rate of 4.3 per 100,000 for women aged 30 to 44 years, giving a relative risk of 2.3. The comparable figures for 1977 and 1982 were 0.8 per 100,000 for women aged ≤19 years and 1.6 per 100,000 for women aged 30 to 44 years, giving a relative risk of 2.0 (Fig. 2). Women aged 20 to 29 years had an interme-

Table I. Legal abortions, abortion-related deaths, and deaths per 100,000 procedures by selected characteristics, 1972-1982

Characteristic	*Deaths*	*Abortions*	*Mortality rate**	*Relative risk (95% CL)*
Age (yr)				
≤19	41	3,394,957	1.2	Referent
20-24	61	3,829,883	1.6	1.3 (0.9-2.0)
25-29	37	2,134,957	1.7	1.4 (0.9-2.2)
30-34	25	1,110,772	2.3	1.9 (1.1-3.0)†
35-39	16	539,562	3.0	2.5 (1.4-4.3)†
40-44	6	190,529	3.2	2.6 (1.1-6.0)†
Race				
White	84	7,731,584	1.1	Referent
Black and other	102	3,469,076	2.9	2.7 (2.1-3.6)†
Parity				
0	72	6,057,216	1.2	Referent
1	32	2,190,233	1.5	1.2 (0.8-1.9)
2	34	1,590,862	2.1	1.8 (1.2-2.7)†
≥3	48	1,362,349	3.5	3.0 (2.1-4.2)†
Region				
West	33	2,748,248	1.2	Referent
North Central	34	2,165,326	1.6	1.3 (0.8-2.1)
Northeast	56	3,339,413	1.7	1.4 (0.9-2.1)
South	63	2.947,673	2.1	1.8 (1.2-2.7)†
Method				
Curettage	82	9,943,057	0.8	Referent
D&E	28	544,689	5.1	6.2 (4.3-9.1)†
Instillation	56	552,238	10.1	12.3 (9.4-16.0)†
H/H	11	24,540	44.8	54.4 (38.4-76.9)†
Gestation (wk)				
≤8	26	5,398,877	0.5	Referent
9-10	33	3,079,493	1.1	2.2 (1.3-3.7)†
11-12	27	1,517,986	1.8	3.7 (2.2-6.1)†
13-15	24	555,730	4.3	9.0 (5.7-14.2)†
16-20	59	529,698	11.1	23.1 (16.9-31.7)†
≥21	14	118,876	11.8	24.5 (15.8-37.9)†

*Rate per 100,000 procedures; CL = confidence limits; D&E = dilatation and evacuation; H/H = hysterectomy and hysterotomy.

†Significant at $p < 0.05$.

diate level of risk from 1972 to 1976, but in 1977 to 1982 they had virtually the same risk as women 19 years old or younger (Fig. 2).

Race. Women of black and other races had a higher risk of mortality than white women during both time periods (Fig. 3). Athough the magnitude of risk decreased for all races, the decline was greatest among whites. As a result, the relative risk for women of black and other races actually increased over the period of time (Fig. 3). From 1972 to 1976, the death-to-case rate for white women was 2.0 per 100,000, whereas for women of black and other races the rate was 5.3 per 100,000, yielding a relative risk of 2.6. From 1977 to 1982, the rate for white women was 0.6 per 100,000, whereas for women of black and other races the rate was 1.8 per 100,000, yielding a relative risk of 3.0.

Parity. The risk of mortality increased with increasing parity (Table I). This finding was observed for both time periods (Fig. 4), although the magnitude of risk decreased over the period of time. From 1972 to 1976, nulliparous women had a death-to-case rate of 2.4 per 100,000, whereas women with three or more previous births had a rate of 4.8 per 100,000, yielding a relative risk of 2.0. From 1977 to 1982, nulliparous women had a rate of 0.7 per 100,000 whereas women with three or more previous births had a rate of 2.3 per 100,000, yielding a relative risk of 3.3.

Gestational age. The risk of dying from legal abortion increased steadily with increasing gestation time. From 1972 to 1982, abortions performed at ≤8 gestational weeks carried the lowest rate of mortality—0.5 deaths per 100,000 procedures (Table I). In comparison, abortions performed at 9 to 10 weeks' gestation had a 2.2 times higher risk of death; the risk increased to 24.5 times at ≥21 gestational weeks (Table I). The risk of death increased with increasing gestation in both time periods, although with time the magnitude of risk in each gestational age group decreased (Fig. 5). The greatest decrease over the period of time occurred in the ≥21-week group (89%), followed by the 13- to 15-week group (74%), the 11- to 12-week group (67%), the 9- to 10-week group (59%), and the 16- to 20-week group (55%). The decrease in mortality risk for abortions performed at ≤8 gestational weeks was 33%.

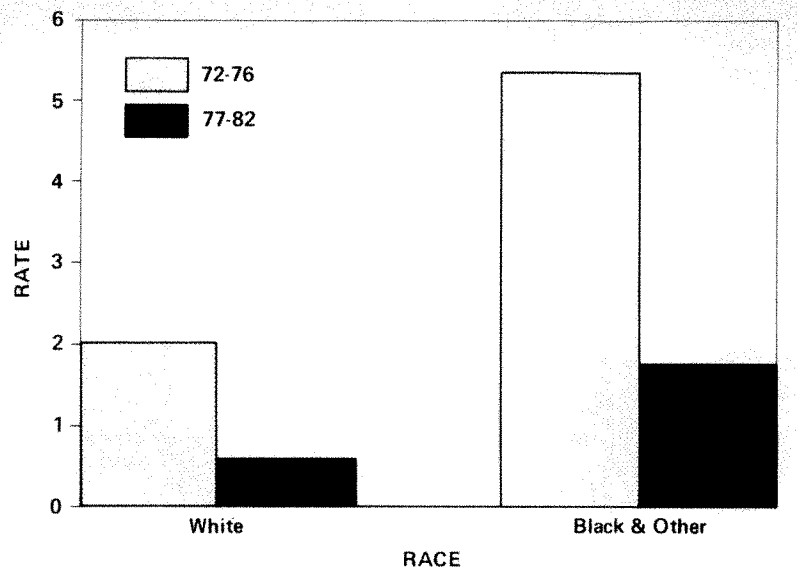


Fig. 3. Legal abortion mortality by race, United States, 1972 to 1976 and 1977 to 1982.

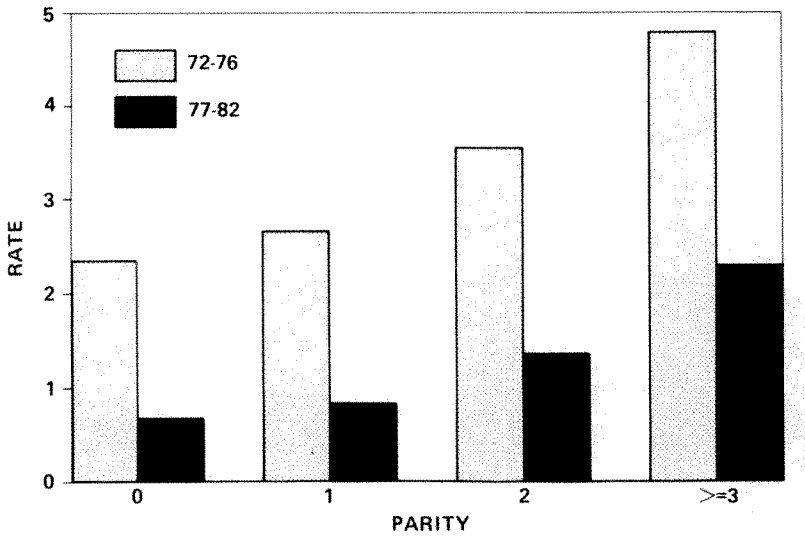


Fig. 4. Legal abortion mortality by parity, United States, 1972 to 1976 and 1977 to 1982.

Geographic region. Women obtaining abortions in the South had the highest death-to-case rate in both time periods. From 1972 to 1976, the lowest rate, 2.5 per 100,000 procedures, was found in the Northeast, whereas the rate in the South was 4.3 per 100,000 (relative risk = 1.7). From 1977 to 1982, the lowest rate, 0.4 per 100,000, was observed in the West, whereas the rate in the South was 1.3 per 100,000 (relative risk = 3.2). Although all geographic areas experienced a drop in abortion mortality over the period of time, the greatest drop (sixfold) occurred in the West (Fig. 6).

Method of abortion. In both time periods, the lowest mortality rates occurred with curettage abortion, whereas the highest rates occurred with hysterotomy and hysterectomy. Over the period of time, the mortality rates for all methods except hysterotomy and hysterectomy decreased. From 1972 to 1976, the death-to-case rate for curettage abortion was 1.3 per 100,000, whereas from 1977 to 1982, the rate was 0.6 per 100,000, a decrease of >50%. From 1972 to 1976, the rate for dilatation and evacuation was 8.5 per 100,000, dropping to 3.2 per 100,000 in 1977 to 1982, a decrease of 62%.

The greatest declines in abortion mortality over the period of time occurred with instillation methods. Saline instillation had a death-to-case rate of 17.8 per 100,000 from 1972 to 1976, whereas the rate fell to 5.1 per 100,000 from 1977 to 1982, a decrease of 71%. Prostaglandin and other instillation had a rate of 13.1

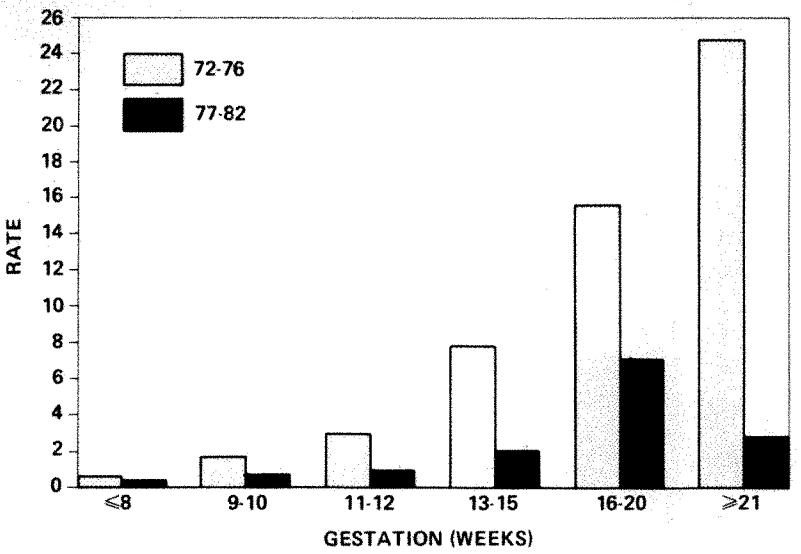


Fig. 5. Legal abortion mortality by gestation, United States, 1972 to 1976 and 1977 to 1982.

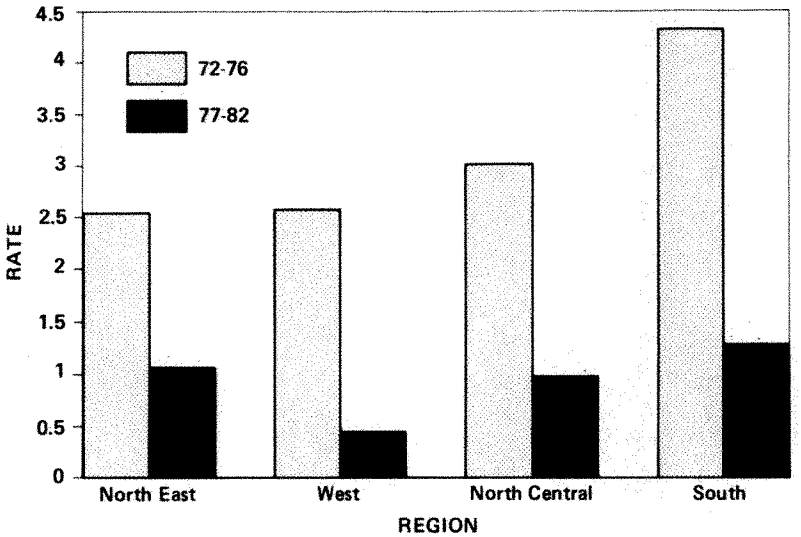


Fig. 6. Legal abortion mortality by region, United States, 1972 to 1976 and 1977 to 1982.

per 100,000 in 1972 to 1976, whereas the rate fell to 3.2 per 100,000 from 1977 to 1982, a decrease of 76%. By contrast, the death-to-case rate associated with abortion by hysterotomy and hysterectomy actually increased from 44.7 per 100,000 in 1972 to 1976 to 58.5 per 100,000 in 1977 to 1982. For all abortion procedures, the risk of death increased with increasing gestational age, and, for each gestational age, the risk of death was lowest for curettage abortion and highest for abortions done by hysterotomy and hysterectomy (Table II).

Causes of death. The leading causes of abortion-related deaths in both time periods were infection, hemorrhage, complications of general anesthesia, amniotic fluid embolism, and pulmonary embolism. Nevertheless, although the number of deaths due to each cause decreased over time, the proportion of deaths resulting from some causes decreased while the proportion due to other causes increased. The proportion of deaths from infections decreased from 26% in 1972 to 1976 to 13% in 1977 to 1982; the proportion resulting from amniotic fluid embolism decreased from 12% to 8%, and the proportion resulting from pulmonary embolism decreased from 11% to 7%. However, the proportion of deaths due to complications of general anesthesia rose from 8% in 1972 to 1976 to 18% in 1977 to 1982, and deaths due to hemorrhage rose from 12% in 1972 to 1976 to 23% in 1977 to 1982. As a result of these shifts, the most frequent cause of death during 1972 to 1976 was infection, followed in number by hemorrhage, amniotic fluid embolism, pulmonary embolism, and complications of general an-

Table II. Legal abortion–related deaths and mortality rates by gestational age and abortion procedure, 1972-1982

Procedure	*Gestataional age (wk)*						*Total*
	≤8	*9-10*	*11-12*	*13-15*	*16-20*	*≥21*	
Curettage	25* (0.5)†	31 (1.0)	26 (1.8)				82 (0.8)
D&E				14 (3.6)	12 (9.5)	2 (10.4)	28 (5.1)
Instillation				5 (5.0)	41 (10.9)	10 (11.7)	56 (10.1)
Hysterotomy and hysterectomy		2 (48.2)	1 (33.1)	3 (62.8)	4 (80.9)	1 (115.1)	11 (44.8)
Total	25 (0.5)	33 (1.1)	27 (1.8)	22 (4.3)	57 (11.1)	13 (11.8)	177‡ (1.6)

*Number of deaths.

†Numbers in parentheses are mortality rates per 100,000 procedures.

‡Six cases done by other procedures and three cases with unknown gestation were excluded.

esthesia. In 1977 to 1982, the most frequent cause of death was hemorrhage, followed in number by complications of general anesthesia, infection, amniotic fluid embolism, and pulmonary embolism.

The leading causes of death varied by age, race, method, region, and gestational age. The racial and age differences, however, disappeared when we adjusted for method of abortion. For curettage abortions the leading cause of death during the 11-year study interval was complications of general anesthesia, whereas for dilatation and evacuation it was infection, for instillation procedures it was amniotic fluid embolism, and for hysterotomy and hysterectomy it was pulmonary embolism. For abortions done at ≤12 weeks' gestation, the leading cause of death was general anesthesia complications, and for abortions done at ≥13 weeks, infection was the leading cause of death. In the Northeast, the leading cause of abortion-related deaths was general anesthesia complications, whereas in the West, South, and North Central regions, the leading cause of death was infection.

Comment

Age, race, gestational age, and abortion method have been repeatedly identified as risk factors for legal abortion mortality and morbidity.[3-7] However, the risk associated with each of those variables has changed over a period of time. Although age has continued to be a determinant of legal abortion mortality, it clearly has become less important. The declining mortality risk among older women may be related to the improved management of infection and pulmonary embolism, the leading causes of death in the older age group between 1972 and 1976. These two causes of death have had the greatest decrease between the two time periods.

A portion of the difference in race-specific mortality may be explained by differences in the gestational age distribution between races. Women of black and other races have a smaller proportion of abortions performed at ≤8 weeks' gestation (the safest interval) and have a larger proportion of abortion at 11 to 12 weeks, as well as during the second trimester. In 1981 10.6% of white women obtaining legal abortions had the abortions during the second trimester, compared with 12.9% of black women and those of other races.[1] Age differences do not explain the mortality differences between the races because only very small differences in the age distribution between the race groups were found. (In 1981 60.9% of white women obtaining legal abortions were ≥20 years old versus 64.4% of black and other women.)[1] We are unable, however, to examine the effects of other factors such as socioeconomic status, parity, marital status, and access to medical care—factors known to be associated with increased mortality.

The effect of parity on abortion mortality may be confounded by age. However, we were unable to examine this because of the lack of denominator data. Nonetheless, the magnitude of the relative risk is much greater with parity than with age, suggesting that age is unlikely to explain the whole difference. The consistently greater mortality in the South may be caused in part by the higher proportion of black women and those of other races who undergo abortion in this region.[1] The differential rates of decline and the variation in the causes of death in the four regions may be related to differences in medical practices. For example, differential decline in the use of general anesthetics or more aggressive management of complications in certain regions could result in variable regional declines in mortality rates.

The very high death-to-case rate with hysterectomy or hysterotomy in comparison with that of other methods may be explained by both the probable higher risk status of women undergoing these procedures and the inherent risk of death from major surgical procedures.[8] The marked drop over a period of time in the mortality rates associated with curettage, dilatation and evacuation, and instillation procedures probably reflects an increase in operator skill resulting from postgraduate training and accumulated experience.

There have been major changes in the leading causes of abortion-related deaths over the time period studied.

These changes result from the changing characteristics of women obtaining legal abortion as well as changing medical practices. Mortality from infection may have declined because of improved treatment, including more aggressive evacuation of retained tissues from the uterus and more aggressive use of antibiotics. Deaths from pulmonary embolism may have declined, at least in part, because of improved infection treatments leading to a reduced likelihood of septic pelvic thrombophlebitis. In addition, improvements in the diagnosis and treatment of pulmonary embolism may have played a role in the decline.

The continued role of general anesthesia deaths in abortion mortality is of concern. In several studies, the use of general anesthesia for abortion, compared with the use of local anesthesia, has been demonstrated to result in both higher morbidity and mortality.[9, 10] Furthermore, it is possible that some of the deaths resulting from hemorrhage are indirectly related to general anesthesia use.[9-11]

Despite the increased risk associated with general anesthesia, its use has remained relatively common. The Alan Guttmacher Institute estimated that in 1982, more than 50% of legal abortions done in Northeastern clinics and physicians' offices (the region with the highest rates of general anesthesia–associated deaths) were performed in facilities offering only general or general and local anesthesia (personal communication). Although this percentage drops to 23.6 in the other three regions, the continued high levels of general anesthesia use is disturbing.

Gestational age was the most important patient-related variable associated with abortion mortality. There was more than a twentyfold difference in mortality between abortions performed at ≤8 weeks and those performed at 16 to 20 weeks. Gestational age was a much stronger predictor of mortality than age, race, parity, or region of the country. The importance of gestational age as a risk factor for mortality is consistent with findings in many studies on morbidity associated with abortion.[6, 7]

Although we cannot be certain that all legal abortion–related deaths from 1972 to 1982 were reported to the CDC, we believe that the use of multiple reporting sources decreases the likelihood that deaths are missed.[12] A study of maternal deaths in the United States between 1974 and 1978 relying on vital records only identified 141 abortion-related deaths, 63 of which were related to legal abortion.[13] In comparison, CDC's surveillance of abortion mortality identified 188 abortion-related deaths during the same period, 92 of which were related to legal abortion. This represents a 46% rise in legal abortion mortality case identification through CDC's surveillance system compared with vital records alone.

We used data from the annual CDC abortion surveillance to provide denominators for death-to-case rates. For most of the variables, denominator data were available for at least 75% of the total number of states reporting abortions. However, two variables, race (68%) and parity (52%), had smaller known denominators. It is unlikely that the characteristics of individuals in the groups for whom the information was reported differed markedly from those in groups for whom the information was not reported. Nevertheless, if such differences existed, they could influence our results.

Before 1978, only univariate data on the demographic characteristics of women obtaining abortions were available from our abortion surveillance. Bivariate data on the method of abortion and gestational age have been available since 1972 and were included in the analysis. Because we were unable to perform multivariate analysis with most of our variables, we cannot discount their potential interactions affecting the death-to-case rates. Nevertheless, we were able to determine the magnitude of risk for each variable and thereby estimate its relative importance.

In conclusion, legal abortion mortality has dropped dramatically over the past decade. The decline was more marked for older women, white women, and women obtaining abortion at later gestational ages. The leading causes of death have changed because of a differential in the rate of decline of various causes of death. Hemorrhage and general anesthesia complications have emerged as the leading causes of death and are of major concern. The increased morbidity and mortality associated with general anesthesia compared with local anesthesia have been repeatedly documented. The use of general anesthesia during the first trimester should be carefully reviewed. As with other surgical procedures, women having abortions should be counseled regarding the pros and cons of local and general anesthesia; use of local anesthesia should be encouraged. General anesthesia should be used only when appropriately trained staff and facilities for monitoring and resuscitation of patients are present. In addition, with the availability of intravenously administered fluids and blood products, it should be possible to prevent most legal abortion deaths caused by hemorrhage.

REFERENCES

1. Centers for Disease Control. Abortion surveillance 1972-1981. US Public Health Service, Atlanta, Georgia: Centers for Disease Control, 1973-1985.
2. Rothman KJ, Boice JD. Epidemiologic analysis with a programmable calculator. Washington, DC: National Institutes of Health 1979; NIH Publication 79-1649.
3. Cates W, Grimes DA, Smith JC, Tyler CW. The risk of dying from legal abortion in the United States, 1972-1975. Int J Gynaecol Obstet 1977;15:172-6.
4. Cates W, Grimes DA, Smith JC, Tyler CW. Legal abortion

mortality in the United States, epidemiologic surveillance, 1972-1974. JAMA 1977;237:452-5.
5. Grimes DA, Kafrissen ME, Oreilly KR, Binkin NJ. Fatal hemorrhage from legal abortion in the United States. Surg Gynecol Obstet 1983;157:461-6.
6. Grimes DA, Cates W. Complications from legally-induced abortion: a review. Obstet Gynecol Surv 1979;34:177-91.
7. Buehler JW, Schulz KF, Grimes DA, Hogue CJR. The risk of serious complications from induced abortion: do personal characteristics make a difference? AM J OBSTET GYNECOL 1985;155:14-20.
8. Grimes DA, Flock ML, Schulz KF, Cates W Jr. Hysterectomy as treatment for complications of legal abortion. Obstet Gynecol 1984;63:457-62.
9. Grimes DA, Schulz KF, Cates W Jr, Tyler CW Jr. Local versus general anesthesia: which is safer for suction curettage abortion? AM J OBSTET GYNECOL 1979;135:1030.
10. MacKay HT, Schulz KF, Grimes DA. Safety of local versus general anesthesia for second trimester dilatation and evacuation abortion. Obstet Gynecol 1985;66:661-5.
11. Grimes DA, Schulz KF. Morbidity and mortality from second trimester abortions. J Reprod Med 1985;30:505-14.
12. Cates W Jr, Smith JC, Rochat RW, Patterson JE, Dolman A. Assessment of surveillance and vital statistics data for monitoring abortion mortality: United States, 1972-1975. Am J Epidemiol 1978;108:200-6.
13. Kaunitz AM, Hughes JM, Grimes DA, Smith JC, Rochat RW, Kafrissen ME. Causes of maternal mortality in the United States. Obstet Gynecol 1985;65:605-12.

Successful pregnancy outcome after cervical dilation with multiple laminaria tents in preparation for second-trimester elective abortion: A report of two cases

Linda Van Le, M.D., and Philip D. Darney, M.D., M.Sc.
San Francisco, California

Two patients at 22 weeks' gestation underwent extensive cervical dilation with laminaria tents for elective abortion but continued their pregnancies instead. Both had normal deliveries. For the unusual patient who chooses not to carry out an abortion initiated with cervical dilation, successful pregnancy is possible and therapeutic intervention such as cervical cerclage seems inadvisable. (AM J OBSTET GYNECOL 1987; 156:612-3.)

Key words: Abortion, cervical dilation, pregnancy outcome

The decision to terminate a pregnancy is often difficult. The maximum gestational age at which termination is possible is determined by state law and institutional policy but usually does not extend beyond 24 weeks. Laminaria tents have been shown to be safe and effective for cervical dilation preceding second-trimester abortion by instrumental evacuation.[1] Two or three sets of multiple laminaria tents are used to achieve 2 to 3 cm of cervical dilation for abortions beyond 18 weeks' gestation.[2] Occasionally women who have decided to terminate a pregnancy change their minds after multiple laminaria tents have achieved considerable cervical dilation. Risks of infection or spontaneous abortion are unknown but are presumed to be high, perhaps without foundation.

From the Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, and the San Francisco General Hospital.
Received for publication July 29, 1986; accepted September 26, 1986.
Reprint requests: Philip D. Darney, M.D., M.Sc., Department of Obstetrics and Gynecology, San Francisco General Hospital, San Francisco, CA 94110.

Two patients who sought abortions at 22 weeks and subsequently changed their minds were followed up to uneventful delivery. In both cases many laminaria tents were placed in the cervix and later removed without complications. These two case histories provide some reassurance that normal pregnancy is possible for women who choose not to carry out an elective abortion already begun with laminaria insertion.

Case reports

Case 1. M. C., a 23-year-old woman, gravida, 4, para 1, elective abortions 2, was a heroin addict with unknown gestational age. She was incarcerated and desired termination of her pregnancy. Sonography showed a biparietal diameter consistent with 22 weeks' gestation. Six medium laminaria tents were placed, followed by six additional tents the next day. Metronidazole was given for trichomonal vaginitis; gonorrhea culture was negative. The patient was released from jail and, despite many urgent messages, refused to come to the clinic. Six days later she returned to explain that the laminaria tents were expelled on the third day. She denied having had passage of fluid from the va-

gina; the membranes were intact, and she had no fever or contractions. The temperature was 97.4° F, and the abdomen was nontender. No laminaria tents were seen and the cervix was long and closed. She still desired an abortion but failed to keep her next appointment.

At 37 weeks' gestation, with no prenatal care, she presented in labor and was rapidly delivered of a healthy 2790 gm female infant with Apgar scores of 8 and 8. Labor and the postpartum course were uncomplicated although the patient left against medical advice.

Case 2. B. M., a 27-year-old woman, gravida 6, para 3, spontaneous abortions 2, presented at 22 weeks' gestation with confirmation by sonography. She desired termination of her pregnancy because of the home situation. She had 17 small laminaria tents placed during the course of 2 days, but on the day of her scheduled abortion she requested that the laminaria be removed because she wished to continue the pregnancy. All the laminaria were removed; the membranes were intact. The cervix was 2 cm dilated and soft. There was no bleeding or fever, and sonographic evaluation showed cardiac motion and adequate amniotic fluid. She was given erythromycin, 500 mg three times a day orally for 7 days. The pregnancy continued without complication and at 40 weeks' gestation she was spontaneously delivered of a 3080 gm male infant with Apgar scores of 9 and 9.

Comment

Approximately 30,000 elective abortions requiring laminaria insertion for uterine evacuation were performed in 1981 according to the Centers for Disease Control. An unknown but probably very small proportion of these women (in our experience about one in 500) decide not to carry out the abortion after the laminaria tents have already been placed. The assumption has been that these pregnancies were in jeopardy because of a high risk of amnionitis and spontaneous abortion. There are, however, no data to support these negative assumptions or to guide clinicians regarding the treatment of patients who ask to have laminaria tents removed from the cervix. These two cases demonstrate that an adverse outcome is not inevitable.

Safe, rapid uterine evacuation for elective abortion in the latter second trimester requires 2 to 3 cm of cervical dilation. Since multiple laminaria tents remained in the cervix for 3 days in one case and 2 days in the other, this degree of dilation was achieved. A comparable spontaneous cervical change at 22 weeks in a desired pregnancy would be worrisome, but in both patients the cervix returned to normal within 1 week. Placement of a cervical cerclage would have been unwise. Prenatal care is especially important for these ambivalent patients. One patient sought care but the other did not.

Patients who decide not to end a pregnancy after having laminaria tents placed may be at higher risk for preterm labor and infection, but these did not occur in the two patients described here. Such vacillation about a critically important decision is fortunately a rare occurrence; after counseling the vast majority of women are firm in their decision to continue or not to continue a pregnancy and follow through accordingly. For the women who change their minds about abortion, removal of laminaria tents, consideration of antibiotic treatment, and referral for prenatal care seems the best course. Management should be expectant, and patients should receive documented warnings about the possibility of amnionitis and premature labor.

REFERENCES

1. Schulz KF, Grimes DA, Carter W Jr. Measures to prevent cervical injury during suction curettage abortion. Lancet 1983;1:182.
2. Hern W. Serial multiple laminaria and adjunctive urea in late outpatient dilatation and evacuation abortion. Obstet Gynecol 1984;63:543.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1987 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 156 (January-June) and Vol. 157 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Long-term observations of cervical cancer

Axel N. Arneson, M.D., and Ming Shian Kao, M.D.
St. Louis, Missouri

A consecutive series of approximately 1100 patients, treated for cancer of the cervix over a span of 45 years, presents an increasingly favorable stage distribution. Emergence of exfoliative cytology as a diagnostic technique accelerates allocation of new patients to Stage I. Cumulative rates of dying from cervical cancer equate improved survival in terms of differences in prognosis and in effectiveness of treatment. Long-term observations bring into focus interval changes affecting the reservoir of individuals remaining at risk, which is maintained in accordance with rates of new patient inflow, as well as withdrawal for all categories of attrition. The several factors determining that balance, including age at diagnosis of cervical cancer, do not fall equally on all clinical stages. Age, a dominant factor determining incidence of second primary malignancies, may also have significance in unraveling the obscure etiology of corporeal malignancies evolving among patients irradiated in the treatment of cervical cancer. (AM J OBSTET GYNECOL 1987;156:614-25.)

Key words: Consecutive series of cervical cancer, long-term observations, early detection and survival, second primary malignancies, corporeal cancer in patients irradiated for cervical cancer

Adaptation of exfoliative cytology into gynecologic practice came as a direct outgrowth of the research of Papanicolaou in estrus of the guinea pig. On expanding investigation to include physiology of the human ovary, he detected malignant cells in vaginal secretions taken from some individuals. Despite that fact, however, his initial report in 1928 met indifferent success as a technique for early detection of cervical cancer.[1] Biopsy and tissue section of overt lesions remained the basis for diagnosis. Not until his report to the Commonwealth Fund in 1943 did the work gain interest and receive support.[2] His associates at the New York Hospital–Cornell Medical Center and the Harvard Group at Boston were among the earliest to initiate programs testing the method by clinical research.[3, 4]

An early consequence of the Papanicolaou test was more frequent detection of unsuspected cellular aberrations. Those encounters intensified interest in the then ongoing quest pursuing the morphologic transition of benign squamous cells of the cervix into precancerous and invasive forms of malignancy. The diverse spectrum of opinion, and the plethora of descriptive terms proposed by clinician and pathologist alike, prompted a "debate" presented as a special session at the 1952 annual meeting of the American Gynecological Society. The primary participants, Hertig and Younge[5] and McKelvey,[6] gave their material in sequence with each making final summation after the closing of open discussion. Proceedings at the session recalled Rubin's first two cases of "incipient cancer" described in 1910, Robert Meyer's prolonged attempt at tracing cellular development in individual patients, and the gradual ill repute falling on "precancerous" as a descriptive adjective. Hertig considered use of "precancerous anaplasia," a term describing the situation "more accurately etymologically speaking than does carcinoma in situ."

The Editorial Committee of the "Annual Report on Results of Treatment in Carcinoma of the Uterus and Vagina" adopted a system in 1950 that included preinvasive cervical cancer as Stage 0. They found usefulness of the category hindered by persistent difficulties in distinguishing dysplasia, carcinoma in situ, and early invasion. That situation prompted exclusion of the class in volumes 11, 12, and 13, but collection of data was resumed following the adoption of more definitive rules for stage grouping at the Vienna Congress of the International Federation of Gynecology and Obstetrics in 1961.[7] Data presented in the annual report included, for each collaborator, a graph showing interval changes in the proportion of patients allocated to the different clinical stages. Volume 15, collated for results obtained from 1954 through 1963, included data submitted by the earliest collaborators and is applicable to a span of 25 or more consecutive years. Despite variations in the experience reported by different institutions, there is evidence of a gradual increase in the proportional allocation of invasive lesions to Stage I. The trend of that

From the Department of Obstetrics and Gynecology and the Mallinckrodt Institute of Radiology, Washington University School of Medicine.
Received for publication July 11, 1986; revised September 29, 1986; accepted October 27, 1986.
Reprint requests: Ming Shian Kao, M.D., Department of Obstetrics and Gynecology, Washington University School of Medicine, 4911 Barnes Hospital Plaza, St. Louis, MO 63110.

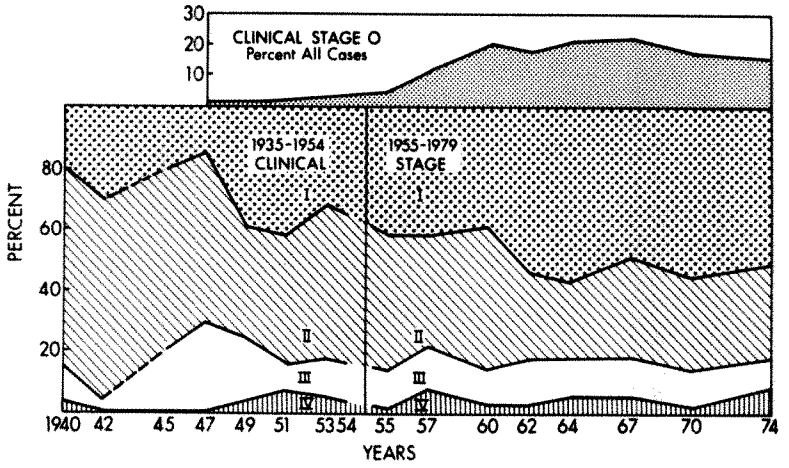


Fig. 1. Composite graph illustrating progressive changes in the distribution of patients into the different stages of clinical advance.

Table I. Distribution of patients into the different stages of invasive disease, and the incidence of carcinoma in situ in two different but consecutive series of cases treated for cervical cancer

	1935-1954		*1955-1979*	
Clinical Stage	*n*	*%*	*n*	*%*
0	10	2.6	119	16.9
I	120	32.0	283	47.2
II	181	48.4	209	35.0
III	62	16.6	78	13.0
IV	11	3.0	29	4.8
Subtotal	374	100	599	100
Total	384		718	

advance appeared to gain momentum after 1950, and thus coincided with the clinical adaptation of exfoliative cytology. That observation prompted us to extend an earlier report dealing with similar data in a consecutive series of private patients. It must be noted, however, that vagary in regional referral practice imposes a selective quality on any change in demography identified with a particular series of cases. There is, however, a measure of credence gained in this instance by the fact that all patients were treated at the same institution and were managed in consultation with the medical staff in attendance over the years in question.

Clinical material

A series of 384 consecutive cases of invasive cervical cancer, treated as private patients for the years 1935 through 1954, has been reported by Arneson and Williams.[8] Stage grouping was in accordance with rules adopted for the League of Nations system and included 10 examples of carcinoma in situ. The first such case was recorded for the year 1947. An additional 718 patients have been added to the series for the years 1955 through 1979. Stage grouping, made in accordance with the International Classification, has necessitated retrospective accommodation in the clinical staging of some cases classified in the earlier years of that period. Among 29 suspected of having early stromal invasion a review of tissue sections qualified 17 for Stage 1A and seven for 1B. The remaining five were transferred to Stage 0, resulting in a final ratio of 599 classified as invasive lesions and 119 as carcinoma in situ.

The allocation of patients into the different stages of tumor advance is shown in Table I for the two groups of consecutive cases. Evidence of earlier detection and diagnosis is made apparent for the 1955 through 1979 series by a significant increase in preinvasive forms and by a greater proportional allocation of invasive lesions to Stage I. The increase falling to Stage I imposes, by necessity, a compensatory decrease in assignment to more advanced forms. That effect appears here to fall largely on Stage II. The combined allocation to Stages I and II approximately 80% of all invasive lesions in each series.

Mean values given in Table I fail to demonstrate interval changes in stage distribution occurring over the time periods in question. Curves for the 1935 through 1954 report were derived by selecting determinate points on a biennial basis.[8] Points fixed at such

Table II. Cumulative data for 599 consecutive patients treated for invasive cervical cancer in the years 1955 through 1979

Years observed	*Year treated*	*No. treated*	*No. at risk*	*Dead—intercurrent disease*		*Lost to observation*		*Dead—cervical cancer*		*Alive and well*		*Cumulative survival (%)*	*New primary tumors*	
				n	%	*n*	%	*n*	%	*n*	%		*n*	%
0-1	1979	4	599	2	0.3	0		45	7.5	552	92.1	92.1	2	0.3
1-2	1978	7	548	2	0.6	3	0.5	47	15.4	496	90.5	83.4	3	0.9
2-3	1977	8	489	7	1.8	2	0.8	38	21.9	442	90.4	75.4	0	
3-4	1976	9	437	5	2.6	2	1.1	23	25.9	407	93.1	70.2	2	1.2
4-5	1975	10	401	10	4.3	0		17	28.9	374	93.2	65.5	2	1.6
5-6	1974	13	369	4	5.0	0		8	30.3	357	96.7	63.4	1	1.8
6-7	1973	14	349	7	6.3	3	1.7	6	31.4	333	95.4	60.5	7	3.1
7-8	1972	15	328	2	6.7	4	2.4	1	31.6	321	98.1	59.2	3	3.6
8-9	1971	18	311	6	7.8	2	2.8	1	31.8	302	97.1	57.5	0	
9-10	1970	19	289	10	9.8	1	3.0	2	32.2	276	95.5	54.9	8	5.3
10-11	1969	20	265	10	11.9	1	3.2	3	32.8	251	94.7	52.0	1	5.5
11-12	1968	30	240	7	13.4	2	3.6	2	33.2	229	95.4	49.6	3	6.1
12-13	1967	21	215	7	15.0	1	3.8	0		207	96.3	47.7	5	7.2
13-14	1966	15	194	8	17.0	1	4.0	0		185	95.3	45.5	3	7.9
14-15	1965	18	178	8	19.0	1	4.3	2	33.7	167	94.0	42.7	3	8.7
15-16	1964	33	161	0		2	4.8	1	34.0	158	98.1	42.0	0	
16-17	1963	37	144	5	20.5	1	5.1	0		138	95.8	40.2	3	9.6
17-18	1962	31	112	2	21.2	2	5.8	1	34.4	107	95.5	38.4	3	10.6
18-19	1961	36	97	4	22.8	0		0		93	95.8	36.8	4	12.2
19-20	1960	30	76	0		0		0		76	100.0	36.8	0	
20-21	1959	45	61	2	24.0	0		0		59	96.7	35.6	0	
21-22	1958	41	47	1	24.7	0		0		46	97.8	34.8	0	
22-23	1957	42	35	0		0		0		35	100.0	34.8	0	
23-24	1956	41	24	1	26.2	0		0		23	95.8	33.3	0	
24-25	1955	42	13	1	28.8	0		0		12	92.3	30.8	0	
		599	5982	111	28.8	28	5.8	197	34.4				53	12.2

short intervals tend to widen fluctuations. A more linear quality is achieved for 1955 to 1979 by selecting points representing approximately equal numbers of patients. A composite graph is shown in Fig. 1. The first determinate point is taken at 1940 because of the small number of patients admitted into the series during early years of the 1935 through 1954 sequence. Declining numbers in the most recent years of the 1955 through 1979 series has been similarly treated by grouping data at year 1974. Explanation should be made for interruptions in curves illustrating stage distribution for years 1942 through 1945. Restrictions of that time not only precluded new admissions but also imposed losses in follow-up procedure. Additional comment on that failure will be given in discussions pertinent to the question.

The composite graph in Fig. 1 shows a sustained increase in the proportional allocation of patients to Stage I throughout the 45-year span, and as noted above, the combined numbers falling to Stages I and II total approximately 80% of all invasive lesions in each series. The graph includes an independent segment showing the proportional allocation to Stage 0. The number of such cases expands slowly following recognition of the first example in 1947. Incidence is elevated to a level near 20% by a more rapid accumulation beginning after year 1955. Concomitant with that increase, the allocation to Stage I appears to take on added momentum. An improved diagnostic technique might be expected to introduce an added flow of early lesions previously left undetected. By the same reasoning it should eventually reduce the reservoir of individuals who might otherwise, by delay and neglect, present more advanced disease at primary diagnosis. This is not made apparent here. If indeed there are inherent factors resisting attempts aimed at achieving earlier diagnosis, they obviously include differences in behavior of individual tumors that can prompt early dispersion or delay the onset of subjective symptoms.

Mention should be made of the "plateau" in preinvasive lesions that follows a rapid gain initiated in the year 1955. On reaching that level the added momentum imparted to the allocation of patients to Stage I appears to be slowed. This concerns the eventual relationship of these two events. If this be a valid epidemiologic observation, it suggests a point of saturation can be reached in the efficacy of exfoliative cytology as a diagnostic instrument. Advance beyond that point would seemingly depend on other methodology or a change in patient demography. This poses need for confirmation in a broader and more comprehensive spectrum of patients. Full significance of the contri-

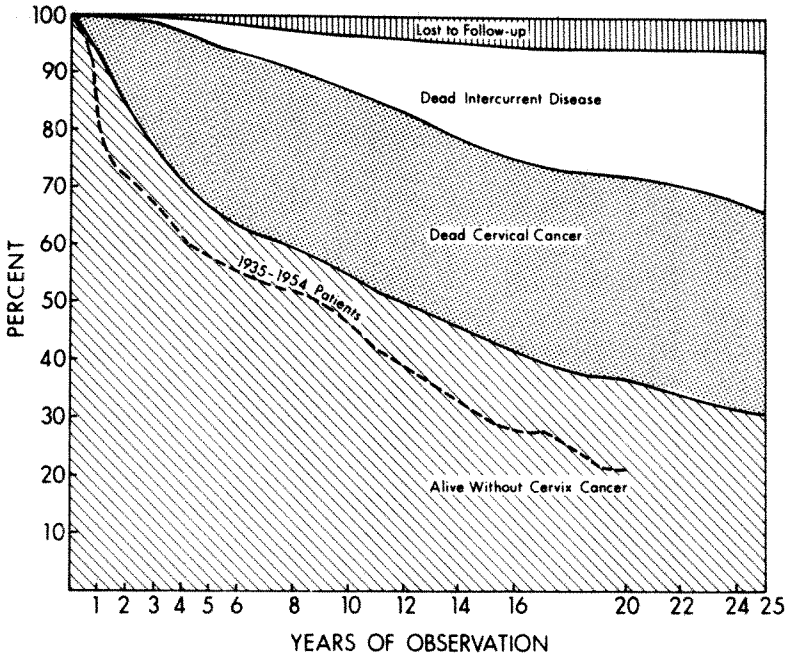


Fig. 2. Curves showing progressive encroachment made by different categories of attrition on the 1955 through 1979 reservoir of living patients. An additional curve presents 20-year cumulative survival for the 1935 through 1954 series.

bution made by exfoliative cytology to cancer diagnosis may never be precisely defined, but in the case of cervical lesions it holds a position of prime importance, enabling earlier detection that is shared only with research unraveling the morphologic transition of benign cells into malignancy.

Clinical results—invasive cancer

The primary treatment employed in both series of invasive cancers has usually been a combination of external irradiation with intracavitary or interstitial applications of radium. Before 1959 the external treatment was administered by 200 kV x rays. Cobalt 60 was used for a short period thereafter, but betatron therapy became the standard procedure after 1962. Exceptions in that management, made by use of primary surgical procedures, are more common in the 1955 through 1979 series.

Actuarial data for the 599 consecutive cases of invasive cancer treated since 1954 are given in Table II. Values show patient survival and specific categories of attrition including deaths resulting from cervical cancer or intercurrent disease, as well as deletions caused by failures in follow-up. In addition to those classified events the incidence of new primary cancers is shown as an independently derived set of values. Longevity is measured from the date of entry into the consecutive series. The point of beginning for patients referred with a confirmed diagnosis of cervical cancer is taken as the date of first examination under our observation. Patients under our observation before the detection of cervical cancer are entered into the series on the date of confirming biopsy. In the development of accumulative data an abstract was prepared for each patient showing follow-up status as of December 31, 1980. A death attributed to cervical cancer occurring 15 months after the point of beginning would find the patient recorded as alive and well at the end of 12 months' observation. On entering the following year at risk status, the patient would subsequently be withdrawn from observation and listed thereafter among other deaths attributed to cervical cancer. The same system is applied in recording attritions identified with either intercurrent disease or failure in follow-up procedure. In the case of deaths caused by cervical cancer, however, the method fails to recognize the time lapse between detection of persistent or recurrent disease, as well as its terminate state. An attempt to reckon with that deficiency was made in the 1935 through 1954 report by initiating an early withdrawal of suspect cases under the heading "alive or dead with cervic cancer."[8] The procedure qualified survival in "absolute" values but, by necessity, compacted cervical cancer deaths into an abnormally abbreviated time span that now presents problems in a direct comparison of clinical results.

Projecting cumulative data in Table II onto curves in Fig. 2 shows more distinctly the proportional encroachment made by different categories of attrition on the reservoir of living patients. Deaths resulting from cervical cancer dominate all events during the first

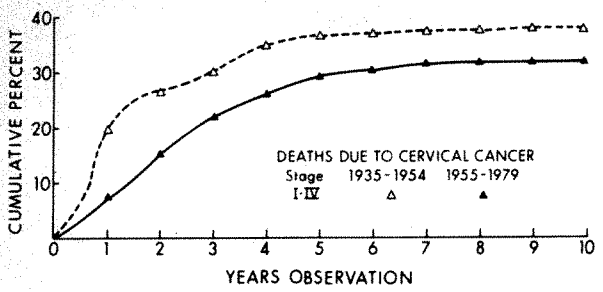


Fig. 3. Curves showing respective 10-year cumulative withdrawal for deaths resulting from cervical cancer in the 1935 through 1954 series and in the 1955 through 1979 series of patients with invasive disease.

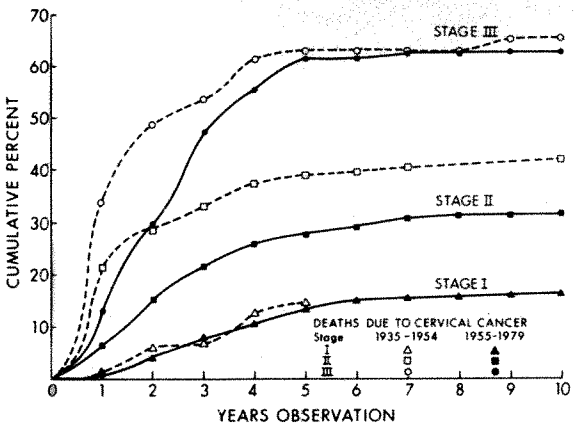


Fig. 4. Curves showing respective 10-year cumulative withdrawal for Stages I, II, and III seen in Table III.

5 years of posttreatment observation. Subsequent encroachment by that category is reduced sharply, however, and at time intervals greater than 10 years the proportional increase falls within two almost parallel curves. Deaths resulting from intercurrent disease, on the other hand, accumulate gradually during early years in follow-up but increase more rapidly as the dominance exercise by cervical cancer begins to fade. At long-term observations the relative proportion of deaths attributed to intercurrent disease may equal, or even exceed, that assigned to cervical cancer. The cumulative loss in follow-up for the 25-year span totals only 5.8%.

An additional curve included in Fig. 2 shows 20-year cumulative survival for the 1935 through 1954 series.[8] The initial rate of fall is exaggerated by the practice then followed in withdrawing patients on the detection of treatment failure. In other respects, however, the curve is seen to fall along a slope 8% to 9% below that for the more recent series until divergence develops near the tenth year of posttreatment observation. Irregularity beyond that point reflects indeterminancy in the long-term status of patients treated before the 1942 through 1945 void in follow-up procedure. That insufficiency precludes comparing long-term results largely affected by deaths resulting from intercurrent disease. Data applicable to the earlier years of posttreatment observations are made more stable by reinstatement of normal procedures in 1946 and by the effectiveness of follow-up during the 7-year period preceding the 1942 void. Since events dominating the period immediately following treatment are largely determined by deaths resulting from cervical cancer, an attempt should be made to reckon the improved survival noted for the 1955 through 1979 series in terms of advance in management or in more favorable curability introduced by an earlier detection of cervical cancer.

The 1935 through 1954 series records withdrawal of 143 examples as treatment failure, or 38.2% of the 374 patients with invasive cancer.[8] In the 1955 through 1979 series of 599 patients a total of 197, or only 32.9%, were withdrawn as cervical cancer deaths. The 5.3% difference suggests more effective management for the recent series. The rate of patient withdrawal, as measured by actuarial data, is tested in Fig. 3 for 10 years posttreatment observation. The curve for 1935 through 1954, as might be expected, shows a more rapid accumulation resulting from the withdrawal of patients on detection of evidence of treatment failure. After 2 years, however, it follows a slope in better conformity with that for 1955 through 1979 but remains at a level representing a greater rate in cumulative withdrawal. A review of these data by Sherry Breaux, M.P.H., of the Oncology Data Center shows the Yates corrected χ^2 equation to be statistically significant at the 0.5 level ($\chi^2 = 4.162$, df = 1, $p = 0.0413$). It should be noted, however, that additional withdrawals are recorded in both series at time intervals of >10 years after treatment. Only one such event is recorded for the 1935 through 1954 series, but as seen in Table II, there are nine additional cervical cancer deaths in the 1955 through 1979 period. Projection of the curves to include those additional examples does not alter their positional relationship but narrows the cumulative difference to only 3.8%. That level, which is lacking statistical significance, fails to fully interpret the difference in overall survival noted in Fig. 2 for the 1955 through 1979 patients.

A difference in overall survival is more effectively explored by reviewing results obtained in different stages of the disease. Actuarial data determined by treatment failures in 1935 through 1954 and by cervical cancer deaths in 1955 through 1979 are given in Table III for Stages I, II, and III. Stage IV is omitted because no patients of that category survived more than 5 years after primary treatment. The "compacting" effect of treatment failure, as a standard of measurement,

Table III. Cumulative data for Stages I, II, and III comparing patient withdrawal for treatment failure in the 1935 through 1954 series and cervical cancer deaths in the 1955 through 1979 series

	Stage I				*Stage II*				*Stage III*			
	1935-1954 (n = 120)		*1955-1979 (n = 283)*		*1935-1954 (n = 181)*		*1955-1979 (n = 209)*		*1935-1954 (n = 62)*		*1955-1979 (n = 78)*	
Years at risk	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
0-1	1	1.0	2	0.7	39	21.5	14	6.6	21	33.9	8	10.3
1-2	6	6.0	10	4.3	14	29.1	18	15.3	9	48.4	15	29.5
2-3	1	6.8	9	7.6	7	33.0	12	21.2	3	53.2	14	47.4
3-4	7	12.6	8	10.5	8	37.4	9	25.6	5	61.2	6	55.1
4-5	2	14.2	7	13.1	3	39.0	4	27.6	1	62.9	5	61.5
5-6			5	15.0	1	39.6	3	29.1			1	62.8
6-7			2	15.7	2	40.9	3	30.7			1	62.8
7-8							1	31.2				
8-9			1	16.1					1	65.4		
9-10			1	16.5			1	31.7				
10-11			3	17.8	1	41.8						
11-12							1	32.3			1	64.1
12-13												
13-14												
14-15			1	18.5			1	33.1				
15-16							1	33.8				
16-17												
17-18							1	34.6				
	17/120	14	49/283	17	75/181	41	69/209	33	40/62	63	50/78	64

is made apparent for 1935 through 1954 patients. Both groups show an increase in withdrawal that is expected to occur with advance in cervical disease. Finally, the number of patients falling into each category is recorded, and Table III presents total withdrawal as a fraction of that value. A review of data for Stage I presents a small margin of difference in end results that perhaps favors the effectiveness of treatment administered the 120 patients of the 1935 through 1954 series. Stage III, on the other hand, presents an uncommon level of equality in the respective results. Only in Stage II is a significant difference to be noted. The margin of difference favoring the more recent series is 9.2 percentage points at 10 years posttreatment. At 18 years, including all cervical cancer deaths in the 1955 through 1979 series, the marginal difference is 7.2 percentage points.

In compliance with the earlier attempt to interpret differences in survival by comparing rates for patient withdrawal, the 10-year data from Table III have been projected on curves shown in Fig. 4. Those for Stage I appear almost superimposed, but for a span of only 5 years. Beyond that time interval the rate for 1955 through 1979 pursues a slow but modest rise. Curves for Stage III present a more expected pattern, which reaches a level of parity at 5 years that is maintained throughout the remaining period. Evidence suggesting a difference in the effectiveness of tumor control is apparent only in the case of Stage II. The rate of patient withdrawal in 1935 through 1954 exceeds that for 1955 through 1979 at all time levels. If there has been an improved survival attributable to treatment alone, the gain is seemingly confined to the intermediate stages of tumor advance. This may be attributable to the higher energies employed for external irradiation and to a more frequent interstitial use of radium during the 1955 through 1979 period. The fact remains, however, that the data at hand do not alone explain the overall gain in survival noted in Fig. 2. That advance is more adequately displayed by the significant increase in patients allotted to Stage I after year 1954.

The dominant factors responsible for an added inflow of more favorable clinical material stem from the emergence of exfoliative cytology as a diagnostic instrument and from the clinical expertise gained in unraveling the morphologic transition of benign squamous cells into malignancy. It is a fortuitous circumstance that temporal relationship of the two series of consecutive patients here in question happens to coincide with impact of those events on clinical practice.

Carcinoma in situ

Prognosis for carcinoma in situ differs from that for invasive cervical cancer because of its relative freedom from deaths resulting from the disease and because of the younger age of most patients. This prompts differences in management and in survival which preclude any attempt to blend the two in a single statement of

Table IV. Cumulative data for 119 consecutive patients treated for carcinoma in situ in the years 1955 through 1979

Years observed	Year treated	No. treated	At risk	Cumulative percent										
				Dead—intercurrent disease		Lost to observation		Dead—cervical cancer		Alive and well		Cumulative survival	New primary tumors	
				n	%	n	%	n	%	n	%	%	n	%
0-1	1979	0	119	2	1.7	0		0		117	98.3	98.3	0	
1-2	1978	1	117	1	2.5	0		0		116	99.1	97.4	2	1.7
2-3	1977	0	115	0		0		0		115	100.0	97.4	1	2.6
3-4	1976	1	115	1	3.4	0		0		114	99.1	96.5	0	
4-5	1975	1	113	1	4.3	1	0.8	0		111	98.2	94.8	0	
5-6	1974	1	110	0		0		0		110	100.0	94.8	0	
6-7	1973	2	109	1	5.2	1	1.7	0		107	98.1	93.0	0	
7-8	1972	1	105	1	6.1	0		0		104	99.0	92.1	0	
8-9	1971	2	103	1	7.0	0		0		102	99.0	91.2	0	
9-10	1970	3	100	0		0		0		100	100.0	91.2	0	
10-11	1969	5	97	1	7.9	0		0		96	98.9	90.2	0	
11-12	1968	7	93	1	8.9	0		0		92	98.9	89.2	0	
12-13	1967	9	86	2	10.9	1	2.9	0		83	96.5	86.0	1	3.6
13-14	1966	6	75	1	12.0	0		0		74	98.6	85.0	1	4.8
14-15	1965	12	70	0		0		0		70	100.0	85.0	1	6.0
15-16	1964	11	59	0		0		0		59	100.0	85.0	0	
16-17	1963	13	49	1	13.7	0		0		48	98.0	83.3	0	
17-18	1962	6	37	0		0		0		37	100.0	83.3	1	8.2
18-19	1961	6	32	1	16.3	0		0		31	96.8	80.7	1	11.0
19-20	1960	8	26	0		0		0		26	100.0	80.7	0	
20-21	1959	11	21	1	20.1	0		0		20	95.2	77.0	0	
21-22	1958	3	10	1	27.8	0		0		9	90.0	69.3	0	
22-23	1957	7	6	0		0		0		6	100.0	69.3	0	
23-24	1956	2	2	0		0		0		2	100.0	69.3	0	
24-25	1955	1	1	0		0		0		1	100.0	69.3	0	
		119	1770	17	27.8	3	2.9	0					8	11.0

end results. In the 1955 through 1979 series of 119 patients a surgical procedure was selected as primary treatment for 93. The usual procedure was abdominal hysterectomy, unless amended to meet requirements for vaginal repair of pelvic supports. Five of the surgically treated patients were selected for management by conization alone. One of that number became pregnant at a later date and was delivered of a mature infant. The remaining patients, 26 in number, were treated by irradiation alone. Indications for selecting that procedure, usually consisting of a single application of intracavitary radium, were determined in accordance with the physical status of the individual in question. The stated age at primary diagnosis of carcinoma in situ fell within a range of 35 to 49 years in 72, or 60% of the 119 patients. Sixteen were younger than 35 years, and 31 were age 50 or older. Average age is 45.4 years in contrast with 51.3 years for the 599 patients with invasive cancer. Both values fall slightly above the corresponding levels prevailing in the 1935 through 1954 period.

Cumulative data provide an effective means for correlating clinical experience in each group. Table IV displays for carcinoma in situ the same categories explored in Table II for invasive cancers of the cervix. Differences in survival are explained by the absence of withdrawals in Stage 0 for deaths due to cervical disease. Since loss due to failures in observation is at a low level in each group, it is obvious that attrition for carcinomas in-situ is largely determined by deaths due to intercurrent disease. The younger age level prevailing for Stage 0 might be expected to less mortality in that category. That expectation is not met by the respective 25-year cumulative values of 28.8% and 27.8%. There is, however, a different rate of cumulative increase. This can be detected by comparing the respective values given in Tables II and IV. The rate of increase for the younger group in Stage 0 falls behind that for invasive cancers; however, at time intervals of 15 or more years after treatment this rate begins to increase and more rapidly expands the accumulation to a total near parity with that reached by older patients with invasive disease. This is in itself an age factor that can be related to the increase in average level imposed by each successive year in follow-up. That effect grows with years in observation and gradually lessens significance of any advantage gained by a difference in average age at diagnosis of the cervical lesion. Data pertinent to the

Table V. Various types of malignancy encountered among 61 new primary cancers, and the distribution of those classes into different stages of cervical disease

Clinical Stage	*Colon*	*Breast*	*Endometrium*	*Ovary*	*Intraoral*	*Lung*	*Lymphoma*	*Leukemia*	*Brain*	*Melanoma*	*Urinary, bladder*	*Stomach*	*No. per stage*
I	8	6	3	3	2	3		2	2	1	1	1	32
II	6	4	1	2		1	1						15
III	1	2	1		1					1			6
IV													0
0	1	4			2		1						8
Total	16	16	5	5	5	4	2	2	2	2	1	1	61

Table VI. Cumulative data pertinent to incidence of new primary cancers

Clinical Stage	*Patients at risk*	*Patient years of observation*	*New primary cancers*		*Cancers per patient year*	*Rate per 100,000*	*Average age at diagnosis*	
			n	*%*			*Cervix*	*New primary*
"0"	119	1770	8	11.0	0.00451	451	45.4	59
I	283	3395	32	16.8	0.00942	942	49.0	62
II	209	2063	15	9.4	0.00727	727	54.7	69
III	78	481	6	7.1	0.01247	1247	56.7	65
IV	29	43	0	—	—	—	59.3	—
Total	718 (1955-1979)	7752	61		0.00786	786	51.3	64
Arneson and Shellhas	874 (1938-1967)	6142	36		0.00586	586	49.0	

Total values are compared with rates obtained for years 1938 through 1967.

occurrence of new primary cancers present the same time relationship. The importance of advancing age is discussed in greater detail in the section dealing with the incidence of second primary malignancy.

Second primary cancers

Probability for encountering a second primary malignancy among patients treated for cancer of the cervix is obviously related to size of the sample and to life span of the survey. The aforementioned review of 718 patients admitted into the series for years 1953 through 1979 has yielded a total of 61 new primary cancers. Six examples of double primary malignancy were disqualified because onset of the additional lesion either antedated or appeared in concurrence with recognition of the cervical tumor. Fifty-three of the 61 new primary cancers evolved among the 599 patients treated for invasive disease of the cervix, and eight were detected in the smaller group of 119 carcinomas in situ. Actuarial data for the two categories, presented in the previously reviewed Tables II and IV, show a 25-year cumulative value of 12.2% for the invasive group and 11.0% for the preinvasive form.

The various types of malignancy encountered among the 61 new primary cancers and distribution of those classes into the different stages of cervical disease is presented in Table V. Variety in type, including dominance of colon and breast disease, does not differ from that expected for women of comparable age in the general population. There is, however, disparity in distribution of the more dominant forms that points up significance of the younger age level prevailing among carcinomas in situ. The 119 patients classified as Stage 0 represent approximately 15% of the entire series but only one of 16 colon cancers evolved in that category, as compared with four of the same number of breast lesions. Average age for Stage 0 falls within the range known to be at risk for mammary cancer but at a level below that identified with equal exposure to colonic and some other forms of malignancy. Disparity can likewise be seen in the number and distribution of new primary lesions occurring in the different stages of invasive cervical disease. Stage I includes 32 of the 61 examples. That density in occurrence falls, however, with each stage of tumor. The rate of decline appears to be in accord with the proportion of patients withdrawn as cervical cancer deaths and the consequent effect of those events on the number of individuals remaining at risk. It is apparent therefore that in addition to factors related to number, time, and age at diagnosis of the cervical tumor, the occurrence of new primary malignancies is affected by the proportional

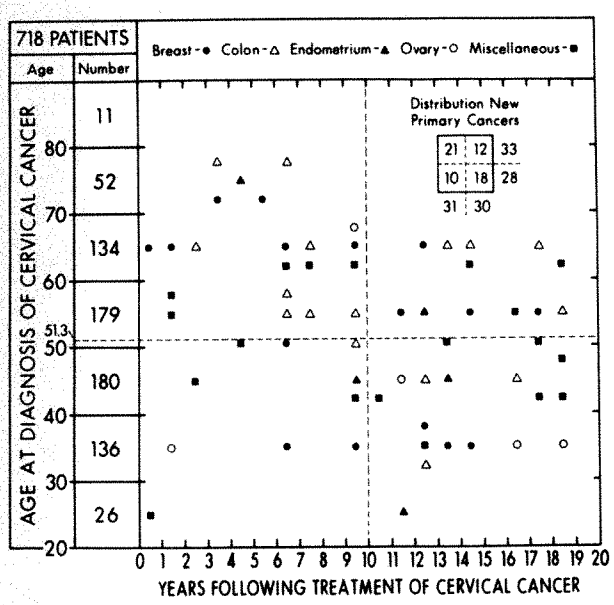


Fig. 5. Scattergram symbolizing posttreatment occurrence of new primary cancers among patients grouped according to age at diagnosis of the cervical lesion.

allocation of patients into the different stages of advance.

Before considering incidence of second primary cancers, mention should be made of two quandaries relevant to the gynecologic varieties that have been encountered. Vulvar lesions are strangely and unaccountably absent. The five endometrial forms evolved in tissues intensively irradiated in treatment of the cervical tumor, and two of them were nonepithelial in type. Rather than now diverting to an examination of the connotation implied by those relationships, we are deferring discussion of the potential for carcinogenic effect until a final summation of results.

An earlier report by Arneson and Schellhas,[9] for patients admitted into the series over years 1935 through 1967, derived incidence from cumulative data that were corrected to state more precisely the number at risk. Correction was made at the beginning of each successive year by subtracting one half of the number dying or withdrawn alive during that time interval. Values so determined tend to even out inequality in risk pattern. A total in "patient years" of observation was taken as the sum of all individuals presented in a state of risk for each time interval. Division of the number of new cancers by that total results in the incidence per patient year, which can then be related to the rate expected per 1000 patients. The more effective level in follow-up procedure, achieved for the 1955 through 1979 span, is believed to warrant deletion of the meticulous correction followed in the earlier report.

Data pertinent to the incidence of new primary cancers are presented in Table VI. Values given for each clinical stage include numerical size of the sample and average age at diagnosis of the cervical lesion. The number of new cancers detected in each category is seen to vary in accordance with size of the sample. Patient years of observation, presented as the total in numbers at risk for each successive time interval, show a similar relationship. It should be noted, however, that ratio of these totals to the numbers of patients in the sample is altered by differences in the rates of withdrawal for cervical cancer deaths. The relative freedom from such withdrawal, prevailing among cases of carcinoma in situ, results in a greater ratio of individuals remaining at risk. This can be expressed by the equation $1770 \div 119 = 14.87$. There are therefore approximately 15 patient years of observation for each individual classified in that category. Application of the same interpretation to different stages of invasive cervical cancer shows deterioration of the quotient to levels of 12, 10, and 6; magnitude of the increase in cumulative withdrawal can be seen in Table III. Among patients of the 1955 through 1979 series the rate is doubled for each stage of tumor advance.

Values representing cumulative percent of new primary cancers are not so precisely related to size of the respective sample. Those given for the different stages of invasive cervical disease are seen to fall in accordance with the rate of patient withdrawal for cervical cancer deaths and are easily correlated with the distribution of examples seen in Table V. The rate of 11.0% for Stage 0 should be compared with the 12.2% for all stages of invasive disease. Both represent the occurrence of such lesions among the number of individuals remaining at risk in each of the two distinct categories of cervical cancer. The eight examples occurring in Stage 0, detected in 1770 patient years of observation, yield the equation $8 \div 1770 = 0.00451$. The 53 examples occurring in all stages of invasive disease, detected in a total of 5982 patient years of observation, yield the equation $53 \div 5982 = 0.00885$.

The ratio of new cancers to the total in patient years of observation, as cited in examples given above, derives cancers per patient year that can be related to incidence expected in the general population. The rate 0.00451 obtained for carcinomas in situ is the lowest of all categories. The corresponding value for invasive lesions, as was determined in accordance with the total in patients remaining at risk, is found to be 0.00885. Incidence levels for the different stages of invasive disease will vary, however, in accordance with the effect of patient withdrawal on the totals in patient years of observation. Stage I, for example, undergoes the least attrition by deaths caused by cervical cancer and thereby presents a relatively greater reservoir of indi-

viduals remaining at risk. The occurrence of new primary cancers is consequently made more frequent, but differences in the reservoir prevailing in other stages can affect frequency as well as incidence. A decline in that reservoir might be expected to lower rates, but severe depletion, as noted for Stage III, may actually elevate incidence as determined for the small number remaining at risk. It should be pointed out, however, that average age of patients is seen to increase with each advance in tumor stage. That increase, perhaps indicative of time elapsing between onset of the lesion and its progression to the stage classed at primary diagnosis, could act to increase probability for encountering new primary cancers. It is a factor of significant importance.

The 61 new primary cancers detected among the 718 patients of the 1955 to 1979 series evolved in a total of 7752 patient years of observation. Incidence for the 25-year interval is 0.00786, or 786 examples to be expected per 100,000 population of comparable age and sex. Average age is found to be 51.3 years at diagnosis of the cervical lesion and 64 years at detection of a second malignancy. The results obtained in the 1938 through 1967 report are included in Table VI and present an incidence of 586 examples expected for 100,000 population. The lesser value for that time interval can be related to the greater burden in loss of follow-up data and to the younger age level prevailing in that period. Both factors would tend to limit the number of new cancers, as well as alter the ratio of that number to the total in patient years of observation. A factor of more positive relevance concerns a difference in the proportional allocation of patients to clinical Stage I.

Dominance of the age factor in determining incidence of second primary malignancies is displayed by the scattergram presented for the 1955 through 1979 series in Fig. 5. Patients are grouped according to the number falling into different age levels at diagnosis of the cervical lesion. The occurrence of second primary cancers is simulated by markers at specific time intervals after primary treatment. The chart is divided into four quadrants by two independently drawn lines. One, placed vertically to indicate a span of 10 years, divides the posttreatment period into two equal time intervals. A second line, drawn horizontally to indicate the 51.3 year level of average age, divides patients into two groups of almost equal numbers. Appropriate markers symbolize the occurrence of new primary cancers, and a small diagram included in the chart summarizes the number of examples detected in each quadrant. The time intervals in question show a yield of 31 second cancers for the decade immediately after primary treatment and 30 such lesions during the second 10-year plan. Distribution is not so precisely equalized at levels above and below average age. A total of 28 second primary cancers evolved among the 342 patients who were younger than the average, as compared with 33 examples detected among the 376 older individuals. The occurrence of new malignancies is lowest during the earlier years after treatment of the youngest patients. Density increases, however, as individuals of that category gain in age with each successive year. That sequence is altered among patients falling into the older age groups because further advance increases patient withdrawal by intercurrent disease. The scattergram indicates that the maximal density for the entire series occurs from the fifth through the fifteenth year after treatment. Possible exceptions to that rule fall in clinical Stages 0 and I, because of the younger age level prevailing in those categories, as well as the relatively greater reservoirs in patients remaining at risk.

The incidence of second primary lesions among patients treated for cancer of the cervix is a complex mechanism primarily determined by age at diagnosis of the cervical disease but also influenced by the proportional allocation of patients into the different stages of clinical advance and by the duration of the time interval in question.

Comment

Long-term observations offer a retrospective view of survival that more closely relates the effects of interval changes to fluctuations in the reservoir of individuals remaining at risk. It is not inappropriate to compare those events with a flowing stream feeding a reservoir or distributary, which maintains a balance determined by a ratio of inflow to that of a continuous drainage through efferent channels. Applying that concept to the particular change introduced at temporal conjuncture of the two groups here in question, conceives the effects of earlier detection and diagnosis as not only increasing the rate of inflow, but also lessening the rate of efferent loss by introducing a more curable pattern. The consequence is an expansion in volume of the "distributary," which reflects itself statistically by registering a proportional advance in survival. The concept can be similarly applied in appraising the average age of patients. All individuals entered into the distributary are subject to withdrawal at one time or another, but the potential does exist for some to remain at that state from the earliest years of the survey to its final closing. Surviving patients are grouped by age in each successive year. The 1935 through 1954 series, initiated by a low volume rate of inflow, expanded the reservoir at risk by a gradual increase in the addition of new patients. On the other hand, the 1955 through 1979 series begins with a reservoir functioning at a favorable level that is subsequently reduced by an eventual decline in

March 1987
Am J Obstet Gynecol

Table VII. Age distribution in two categories of uterine malignancy occurring at different intervals following treatment for cervical cancer

Histology	No. of patients	Age (yr)		Interval (yr)
		At cervical primary	At second primary	
Adenocarcinoma	5	56.6	68	11.4
Sarcomatous	4	34.7	55	20.3
Total	9	47	62.2	15.2
1955-1979	61	51.3	64	12.7

Data for 1955 through 1979 are included as a reference standard.

the number of new admissions. The consequence here is a difference in average age. The level for the first group is weighted toward the greater number of individuals admitted in concluding years of the series, whereas that for years 1955 through 1979 is weighted toward those who have been in the study longer. A difference in average was noted for the two groups in discussion of the significance of age as a factor determining incidence of new primary cancers. Whatever the importance of age, it is a factor found to vary in accordance with that prevailing at diagnosis of the cervical lesion, the number of years included in the survey, and specific categories of patient withdrawal which affect the reservoir of individuals remaining at risk.

Among 61 new primary cancers detected in the 25-year review of the 1955 to 1979 series, there are five examples of endometrial malignancy occurring 4 to 14 years after a program of irradiation applied in the treatment of invasive cancer of the cervix. That number of cases, presenting an incidence of 0.00083, appears to be in line with other histologic types recorded in Table V. It is the evolvement of such lesions in tissues so profoundly altered by postirradiation atrophy and sclerosis that is a point of concern. Two additional examples have been detected, but at time intervals beyond limits defined by this report. Both were diagnosed 22 years after treatment of a patient in 1961 and of another in 1963. Our first encounters with second primary cancers of this order occurred in the 1935 to 1954 series. A 69-year-old patient admitted in 1949 developed a Grade IV adenocarcinoma of the endometrium 8 years later. A young patient aged 30 years treated in 1947 presented with endometrial stromal sarcoma after an interval of 33 years.

These nine examples are grouped in an attempt to better define circumstances surrounding their origin. Stage distribution is in accordance with the proportional distribution of patients—six in Stage I; two in Stage II; and one in Stage III. The second primary lesions include five endometrial adenocarcinomas, two of which are poorly differentiated tumors and four are of a sarcomatous quality. In the more complex variety two have been diagnosed as mixed müllerian, one as carcinosarcoma, and one as an endometrial stromal sarcoma. This wide range in histology is associated with age distributions and with time intervals required for evolvement of new primary cancers, which differ from the average values established for the 61 examples detected in the 1955 to 1979 series. Among the five examples of adenocarcinoma of the endometrium, the stated age at diagnosis of the cervical lesion ranged from 43 to 72 years, with intervals between that diagnosis and detection of the second primary cancer ranging from 4 to 22 additional years. The four patients presenting with second lesions of a sarcomatous type are from a much younger age level and demonstrate a significant increase in interval years. Of that number the respective ages at diagnosis of the cervical tumor were 25, 30, 35, and 49 years. Intervals preceding evolvement of the second primary cancer ranged from 12 to 33 years.

Average values in age distribution at diagnosis of the first and second primary cancers are shown in Table VII for the two categories of endometrial disease. The difference in those values is taken as the interval in years. Corresponding data for 61 new primary cancers detected in the 1955 through 1979 series are included to serve as a reference standard. The five examples of diagnosed adenocarcinoma of the endometrium are in reasonably good accord with levels established for the larger sample. The slight decrease in interval years is adequately explained by the modest increase in average age at diagnosis of the cervical cancer. Values for the four second primary uterine cancers of a sarcomatous type deviate sharply from those given as a standard, with the difference noted chiefly in the much younger age level at diagnosis of the cervical primary. Average age at detection of the second primary is elevated to a more consistent level by a significant increase in interval years. The effects of that increase is also apparent in total averages for all nine examples of endometrial disease.

The circumstances here in question suggest a condition most apt to occur in patients younger than the average age expected for cervical cancer. The detection of sarcomatous types of lesions in tissues so long before exposure to high-dose levels of radiation points up the possibility for carcinogenic effects attributable to that

exposure. Repercussions of a malignant nature have been correlated with the exposure of young individuals to modest amounts of irradiation. More intensive therapeutic procedures, applied in the treatment of adults, have resulted in persistent dermal effects associated with malignant degeneration. The common denominator in such events may well be a prolonged interval between exposure and the eventual occurrence of a new malignancy. The issue is an interesting conjecture that should be pursued. Even if the hypothesis is proved to be correct, the low incidence so far noted is not one that would necessarily deprecate the usefulness of irradiation in the treatment of cervical cancer.

REFERENCES

1. Papanicolaou GN. Third Race Betterment Conference. 1928:538 (reprinted in CA 1973;23:174).
2. Papanicolaou GN. Diagnosis of cancer by vaginal smears. New York: The Commonwealth Fund, 1943.
3. Achenbach RR, Johnstone RE, Hertig AT. The validity of vaginal smear diagnosis in carcinoma in situ of the cervix. AM J OBSTET GYNECOL 1951;61:385.
4. Hinsey JC. George Nicholes Papanicolaou, M.D., Ph.D., D.Sc. Acta Cytol 1962;6:483.
5. Hertig AT, Younge PA. A debate: What is cancer in situ of the cervix? Is it the preinvasive form of true carcinoma? AM J OBSTET GYNECOL 1952;64:807.
6. McKelvey JL. Carcinoma in situ of the cervix: a general consideration. AM J OBSTET GYNECOL 1952;64:825.
7. Kottmeier HL, ed. Annual report on the results of treatment in carcinoma of the uterus and vagina: statements of results obtained in 1948 to 1957, inclusive. vol. 13. Vienna Congress of the International Federation of Gynecology and Obstetrics. (Collated in 1963.)
8. Arneson AN, Williams CF. Long-term follow-up observations in cervical cancer. AM J OBSTET GYNECOL 1960; 80:775.
9. Arneson AN, Schellhas HF. Multiple primary cancers in patients treated for carcinoma of the cervix. AM J OBSTET GYNECOL 1970;106:1155.

The value of endocervical curettage in the management of the patient with abnormal cervical cytologic findings

David A. Grainger, M.D., Daniel K. Roberts, M.D., Ph.D., Max M. Wells, M.D., and Douglas V. Horbelt, M.D., M.A.
Wichita, Kansas

Patients with adequate colposcopic examinations and dysplasia on the endocervical curettage specimen usually undergo further diagnostic evaluation. This study evaluated 712 patients and found the frequency of positive endocervical curettage specimens to be 17.6%. Cervical conization or hysterectomy specimens were examined in 66 cases with a positive endocervical curettage. Of these, 69.7% had histologic evidence of discontinuous dysplasia in the endocervical canal. The endocervical curettage specimens were grouped by the percentage of endocervical epithelium that was dysplastic: group 1, <10%; group 2, 10% to 50%; group 3, >50%. Confirmed histologic endocervical involvement was 80%, 56%, and 60%, respectively. The involvement of the endocervical canal also increased with increasing grade of cervical intraepithelial neoplasia. Of the 66 cases examined with positive endocervical curettage specimens, 62.1% had grade 3 cervical intraepithelial neoplasia on the ectocervix. Seven patients had positive endocervical margins, with six of these occurring in grade 3 cervical intraepithelial neoplasia lesions. Two unsuspected cases of cervical malignancy were identified by endocervical curettage. (AM J OBSTET GYNECOL 1987; 156:625-8.)

Key words: Endocervical curettage, abnormal cervical cytologic findings, cervical intraepithelial neoplasia

The management of the patient with abnormal cervical cytologic findings includes colposcopic examination of the cervix.[1-5] An adequate colposcopic examination is one in which (1) the entire transformation zone and the squamocolumnar junction are completely seen; (2) the entire extent of the lesion is seen; (3) the histologic, cytologic, and colposcopic impressions correlate within one grade; and (4) the endocervical curettage is negative for dysplastic cells.

An endocervical curettage is positive when dysplastic tissue is present. If failure to meet criteria 1, 2, or 3 is

From the Department of Obstetrics and Gynecology at Wesley Medical Center, University of Kansas School of Medicine at Wichita.
Received for publication June 30, 1986; accepted September 26, 1986.
Reprint requests: David A. Grainger, M.D., Wesley Medical Center, Department of Obstetrics and Gynecology, 550 North Hillside, Wichita, KS 67214.

Table I. Relationship between quantity of dysplasia in endocervical curettage specimens and disease in the endocervical canal

	Amount of dysplasia in endocervical curettage specimen (%)	*Patients with dysplasia in endocervical canal*	
		n	%
Group 1	<10	29/36	80
Group 2	10-50	14/25	56
Group 3	>50	3/5	60
Total		44/66	69.7

the reason for an inadequate colposcopic examination, the finding of dysplasia in the endocervical curettage is predictably high, with values ranging from 50% to 80%.[6] In adequate colposcopic examinations, the frequency of dysplasia in the endocervical curettage is reported to be betwen 8% and 18%.[7] Patients with positive endocervical curettage specimens and an otherwise adequate colposcopic examination are still at risk for the presence of neoplastic change in the endocervical canal. Evaluation of the endocervical canal may include conization of the cervix. The purpose of this study is to assess the correlation of a positive endocervical curettage with the presence of intraepithelial or invasive neoplastic disease in the endocervical canal.

Methods

Data were obtained for this study by reviewing the reports of colposcopic examinations performed at a university-affiliated colposcopy clinic from July 1982 through July 1984. During this 2-year period, clinicians trained and supervised in colposcopy by a gynecologic oncologist examined 838 patients to evaluate abnormal cervical cytologic findings. Pregnancy patients (96) and those with inadequate colposcopic visualization (30) did not undergo endocervical curettage and were excluded. Therefore 712 patients were available for analysis.

A total of 126 patients had an endocervical curettage that was considered positive, that is, one in which dysplastic tissue was present. A gynecologic pathologist reviewed all positive endocervical curettages for which surgical specimens were available (66/126). The amount of dysplastic tissue present in the endocervical curettage was divided into three groups: group 1 had <10% dysplasia in the endocervical specimen; group 2 had from 10% to 50%; group 3 had >50%.

Sixty-six cervical conization or hysterectomy specimens were reviewed for histologic diagnosis. The other 60 cases were performed at other institutions, and the specimens were not available for review. The endocervical canal and endocervical margin of each curettage, conization, and hysterectomy specimen were examined for the presence or absence of dysplasia. Endocervical canals that were denuded of epithelium were unevaluable and therefore considered to be negative. The final histologic grade of cervical intraepithelial neoplasia was correlated with the presence or absence of endocervical involvement.

Results

Evaluation was carried out for 712 patients with abnormal pap smears who underwent colposcopic examination. All cases had an endocervical curettage performed. Of these, 17.6% (126/712) had dysplasia present in the endocervical curettage. Of the 126 cases with a positive endocervical curettage, 66 surgical specimens of either conization or hysterectomy were available for review.

Table I shows the distribution of cases with respect to the quantity of dysplasia present in the endocervical curettage specimen. Group 1 consisted of 36 cases (63%) that had <10% dysplasia present in the specimen. Group 2 contained 25 patients (30%) who had dysplasia in between 10% and 50% of the specimen. Group 3 had five (6.6%) patients with >50% dysplasia.

Table I further depicts the relation between quantity of dysplasia and the presence or absence of endocervical dysplasia in the surgical specimen; 80% of group 1, 56% of group 2, and 60% of group 3 had dysplasia in the endocervical canal. Overall, 69.7% (46/66) of the patients with a positive endocervical curettage specimen had histologic evidence of dysplasia in the endocervical canal.

The presence of dysplasia in the endocervical canal directly correlates with the final histologic grade of cervical intraepithelial neoplasia on the ectocervix (Table II). Grade 3 cervical intraepithelial neoplasia was the final histologic diagnosis for 62.1% of those patients with a positive endocervical curettage specimen; grade 2 cervical intraepithelial neoplasia for 25.7%; and grade 1 cervical intraepithelial neoplasia for 9%. Table II also shows that 85% (6/7) of the cases with the positive endocervical margin occurred in those with grade 3 cervical intraepithelial neoplasia lesions.

Among the patients with a positive endocervical curettage for whom surgical specimens were available, two unsuspected cases of invasive carcinoma were identified. Neither patient had the diagnosis made by colposcopically directed cervical biopsy. Conizations prompted by a positive endocervical curettage revealed the invasive lesions. One patient had adenosquamous carcinoma, and the other had squamous cell carcinoma.

Comment

Evaluation by colposcopy of the patient with an abnormal cervical cytologic report places the burden of diagnosis on the colposcopist. If all patients with abnormal Papanicolaou smears underwent conization of

Table II. Correlation of histologic grade with positive endocervical curettage and endocervical margin

Final histologic grade	*Patients with dysplasia in endocervical curettage specimen (n = 66)*		*Endocervical canal positive for dysplasia*		*Positive endocervical margin*
	n	%	*n*	%	
Grade 1 cervical intraepithelial neoplasia	6	9	3/6	50	0
Grade 2 cervical intraepithelial neoplasia	17	25.7	12/17	70.6	1
Grade 3 cervical intraepithelial neoplasia	41	62.1	29/41	70.7	6
Invasive squamous cell carcinoma	2	3.3	2/2	100	NA*

*Not applicable.

the cervix, then the histologic interpretation would be all that is necessary to divide the patients who need further therapy from those who can be managed in a conservative fashion. However, not only is conization of the cervix a procedure that includes significant risk and expense, but it is also not practical to apply it as the first level of evaluation in all patients. The colposcopist's examintion produces an appropriate screen for cases that can be handled conservatively.

This series demonstrates the value of the endocervical curettage as a component of the colposcopic examination. First, it accurately reflects the presence of dysplastic epithelium in the endocervical canal, even when there is no other colposcopic evidence of involvement. In 69.7% of the cases with a positive endocervical curettage, discontinuous dysplasia was demonstrated to be present in the endocervical canal on the conization or hysterectomy specimen. Approximately 20% of specimens showed only denuded endocervical canals, which presumably were caused by the endocervical curettage. These were considered negative but may represent an even greater degree of correlation between endocervical curettage and the histology of the endocervical canal than we could confirm.

This study found a lack of correlation between the actual amount of dysplasia on an endocervical curettage specimen and its accuracy in predicting the presence of dysplastic epithelium. Our study clearly indicates that a small amount of dysplasia should evoke the same response as a large amount. Pathology reports that state "only a small amount of dysplasia noted on endocervical curettage" should not dissuade the practitioner from further evaluation of possible abnormalities in the endocervical canal.

In this study, two patients who met all the criteria for an adequate colposcopic examination except the presence of dysplasia on endocervical curettage specimen were found to have invasive carcinoma in the endocervical canal. These lesions would have been overlooked and allowed to progress had the endocervical curettage results not alerted the clinicians to the possibility of endocervical pathology. These cases represented 3.3% of the cases that were histologically and cytologically reviewed, which is slightly higher than others have reported.[7] This percentage may be statistically unusual; but even if the rate were half, it would still justify the use of the endocervical curettage. One recent large study identified 15 cases of invasive disease. Three of these had adequate colposcopic examinations except for dysplasia (two with grade 2 and one with grade 3 cervical intraepithelial neoplasia) in the endocervical canal.[8] These patients were found in an early stage of disease at a time when effective therapy could be used for cure rather than merely for palliation.

The value of the endocervical curettage in the colposcopic examination is therefore twofold. First, it accurately provides a statement concerning the involvement of the endocervical canal with dysplasia so that conservative therapies such as cryotherapy and laser can be used appropriately. Second, it provides a means for the identification of occult invasive lesions in the endocervical canal. Applied and responded to appropriately, the endocervical curettage should significantly decrease the rate of invasive processes erroneously diagnosed as intraepithelial. Therefore, unless it is clinically contraindicated, we recommend that endocervical curettage be a routine part of colposcopic evaluations for abnormal cytologic findings.

REFERENCES

1. American College of Obstetricians and Gynecologists. Cervical cytology: evaluation and management of abnormalities. Technical bulletin no. 81. Washington DC, 1984.
2. Townsend DE, Ostergard DR, Mishell DR, Hirose FM. Abnormal Papanicolaou smears. AM J OBSTET GYNECOL 1970;108:429.
3. Creasman WT, Clarke-Pearson DL, Ashe C, Weed JC. The abnormal Pap smear—what to do next? Cancer 1981; 48:515.
4. Stafl A, Mattingly RF. Colposcopic diagnosis of cervical neoplasia. Obstet Gynecol 1973;41:168.
5. Ronk DA, Jimerson GR, Merrill JA. Evaluation of abnormal cervical cytology. Obstet Gynecol 1977;49:581.

6. Shingleton HM, Gopre H, Austin JM. Outpatient evaluation of patients with atypical Papanicolaou smears: contribution of endocervical curettage. AM J OBSTET GYNECOL 1976;126:122.
7. Drescher CW, Peters WA III, Roberts JA. Contribution of endocervical curettage in evaluation of abnormal cervical cytology. Obstet Gynecol 1983;62:343.
8. Hatch KW, Shingleton HM, Orr JW, Gore H, Soong S. Role of endocervical curettage in colposcopy. Obstet Gynecol 1985;63:403.

Atypia in cervical cytology as a risk factor for intraepithelial neoplasia

Joel S. Noumoff, M.D.
Philadelphia, Pennsylvania

All Papanicolaou smears obtained during the years 1977 to 1982 were reported by a descriptive cytologic interpretation instead of a numerical report. All smears showing "atypia" were reviewed and found to fall into one of three categories: inflammatory atypia, squamous atypia, and endocervical atypia. Patients with the latter two categories and those with persistent inflammatory atypia after specific therapy underwent colposcopy and directed biopsies if indicated. Colposcopically directed biopsies revealed that 29% of patients with atypical Papanicolaou smears had underlying cervical intraepithelial neoplasia. There was no statistically significant difference in the incidence of underlying cervical intraepithelial neoplasia among patients in each of the three categories of atypia. Of those with intraepithelial neoplasia, 35% had lesions of greater severity than grade 1 cervical intraepithelial neoplasia. We conclude that all patients with squamous, endocervical, and persistent inflammatory atypia on Papanicolaou smear should undergo colposcopic evaluation to rule out intraepithelial neoplasia. (AM J OBSTET GYNECOL 1987;156:628-31.)

Key words: Atypia, dysplasia, intraepithelial neoplasia, Papanicolaou smear

Since Papanicolaou[1] first described the use of exfoliative cytology for the detection of carcinoma, the role of cervical sampling in the routine screening for neoplasia of the uterine cervix has become recognized and well accepted throughout the world. In response to recent controversy regarding the use of Papanicolaou's original classification, the Terminology Committees of the American Society of Cytology and the International Academy of Cytology have recommended using a descriptive cytologic interpretation instead of a numerical report.[2]

Presently most cytopathologists report samples previously designated class II by using descriptive phrases that include the word atypia. Atypical smears are further subdivided into three categories: inflammatory atypia, squamous atypia, and endocervical atypia. The "atypical Papanicolaou smear" presents the gynecologist with a diagnostic dilemma. Although it does not indicate the presence of malignant cells, neither does it connote a normal state. At this time, some clinicians ignore the atypical smear, whereas others treat the patient with various antibacterial agents in the hope that the next cervical sample will be completely normal. It was therefore our decision to embark on a study to ascertain the clinical significance of cervical atypia reported from a Papanicolaou smear to help us to determine appropriate subsequent patient management.

From the Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Albert Einstein Medical Center, Northern Division.
Presented to The Obstetrical Society of Philadelphia, December 12, 1985.
Received for publication March 21, 1986; revised July 11, 1986; accepted September 10, 1986.
Reprint requests: Dr. Joel S. Noumoff, Director, Division of Gynecologic Oncology, Albert Einstein Medical Center, Northern Division, York and Tabor Roads, Philadelphia, PA 19141.

Material and methods

For a 6-year period women with Papanicolaou smears reported as showing squamous or endocervical atypia or whose smears revealed persistent inflammatory atypia (8.7% of all the patients with inflammatory atypia) despite specific antivaginitis therapy (for example, for *Candida, Gardnerella,* or *Chlamydia*) were referred to the Dysplasia Clinic. During the time of this study, all Papanicolaou smears were obtained by moistened cotton-tipped applicators used to collect endocervical, exocervical, and vaginal pool samples. Each sample was directly smeared on a clean slide and immediately sprayed with fixative. After staining, the slides were screened by cytotechnologists and evaluated

Table I. Results of 67,260 routine papanicolaou smears performed in a 6-year period

Category	*No.*	*%*
Negative	43,584	64.8
Atypia		
Inflammatory	19,842	29.5
Squamous	1,816	2.7
Endocervical	336	0.5
Dysplasia (mild-severe)	1,345	2.0
Cervical intraepithelial neoplasia (carcinoma in situ)	202	0.3
Invasive cancer	135	0.2
Total	67,260	

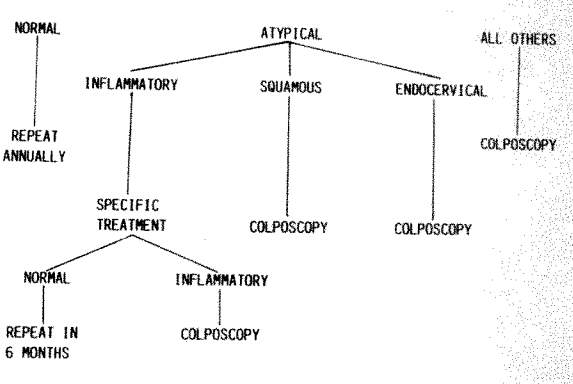


Fig. 1. Recommended follow-up of cervical cytologic results.

for cellular adequacy. Abnormal slides were then reviewed by one of two cytopathologists.

At the Dysplasia Clinic a repeat Papanicolaou smear was obtained, and the cervix was then washed with a 3% acetic acid solution followed by colposcopic assessment of the epithelium by one of two colposcopists. An endocervical curettage was performed, and directed biopsies were obtained from areas of abnormality.

We define each of the three categories of atypia as follows. Inflammatory atypia includes morphologic changes of cells associated with specific infections, at times used in the same sense as repair (that is, reparative squamous epithelium in response to injury or ulcer, and so forth). In squamous atypia, the cytologic features are variable. The cells may be enlarged and may have an increased nuclear/cytoplasmic ratio and a minimally irregular nuclear membrane. At times the chromatin is smoothly granular and evenly dispersed but also may exhibit pyknosis. Binucleation is occasionally a feature. Chromocenters are at times numerous and sometimes single small nuclei may be noted. These cells are beyond the inflammatory atypia category previously described but do not meet the light microscopic criteria of the dysplastic (neoplastic) cell. Endocervical atypia includes abnormalities of glandular cells that may represent the glandular analogue of squamous atypia. Such changes are often associated with an endocervical polyp and endocervical hyperplasia, such as strikingly enlarged nuclei with numerous chromocenters and one or more large, conspicuous nuclei. Multinucleated endocervical cells may occur, and mitoses are not infrequent.

All of the charts from the Dysplasia Clinic of the Bellevue Hospital Center from 1977 through 1982 were reviewed. All patients with squamous, endocervical, or persistent inflammatory atypia were included in the study. Any woman with a previous history of cervical intraepithelial neoplasia or invasive cancer was excluded from the study. Patients who were pregnant at the time the atypical smear was obtained were excluded as were patients whose smears were positive for, or suggestive of, condylomatous or koilocytotic atypia. All abnormal slides were reevaluated for accuracy by a single cytopathologist before inclusion in this study. The data were tabulated and tested for statistical significance by χ^2 analysis.

Table II. Distribution of colposcopically directed, biopsy-verified cervical intraepithelial neoplasia among cervical atypia groups determined by papanicolaou smear

Papanicolaou smear result	*Total No.*	*Cervical intraepithelial neoplasia*	
		n	*%*
Squamous atypia	286	77	27
Inflammatory atypia	72	23	32
Endocervical atypia	17	8	47
Total	375	108	29

Results

A total of 67,260 cervical smears were done during the 6 years included in this study. The results are shown in Table I. From this patient population, after review of charts and pertinent Papanicolaou smear and biopsy slides, 375 patients evaluated in the Dysplasia Clinic for squamous, endocervical, or persistent inflammatory atypia were deemed suitable for inclusion in this study. The mean age of the patients with cervical atypia was 30.2 years. There was no significant age difference between patients whose cervical smears were grouped in each category of atypia.

Colposcopically directed biopsies revealed that 108 (29%) of the women with atypical Papanicolaou smears had underlying cervical intraepithelial neoplasia (Table II). The incidence of cervical intraepithelial neoplasia did not significantly differ between each of the atypia groups. The severity of the intraepithelial neoplasia did not differ significantly between the types of atypia with which the patients initially presented (Ta-

Table III. Distribution according to severity of intraepithelial neoplasia among cervical atypia groups determined by papanicolaou smears

Papanicolaou smear result	*Total No.*	*Mild dysplasia*		*Moderate dysplasia*		*Severe dysplasia*		*Carcinoma in situ*	
		n	%	*n*	%	*n*	%	*n*	%
Squamous atypia	286	51	18	14	5	6	2	6	2
Inflammatory atypia	72	14	19	5	7	4	6	—	—
Endocervical atypia	17	5	29	1	6	2	12	—	—
Total	375	70	19	20	5	12	3	6	2

Table IV. Distribution of 375 patients with repeat papanicolaou smears and colposcopically directed cervical biopsies

Cervical histologic result	*Repeat papanicolaou smear result*					
	Negative		*Atypia*		*Cervical intraepithelial neoplasia*	
	n	%	*n*	%	*n*	%
Cervical intraepithelial neoplasia	10	9	90	83	8	8
Normal	59	22	203	76	5	2

ble III). However, it is important to note that 38 (35%) of the 108 patients with intraepithelial neoplasia had lesions of greater severity than grade 1, and 17% had grade 3 (12 patients had severe dysplasia and six patients had carcinoma in situ). Analysis of the results tabulated from repeat Papanicolaou smears taken at the time of colposcopically directed cervical biopsies (Table IV) revealed that 92% of patients histologically demonstrated to have cervical intraepithelial neoplasia had cytologic classification as either negative (9%) or atypical (83%) on repeat smear. Conversely, only 2% of the patients with normal cervical biopsy findings were cytologically classified as having cervical intraepithelial neoplasia.

Comment

This study is the first to correlate atypical Papanicolaou smears with cervical biopsies guided by colposcopic direction. Our definition of atypia excludes dysplastic cytologic smears and those demonstrating any evidence of condylomatous or koilocytotic changes. The data suggest that patients with atypical smears (squamous, endocervical, or persistently inflammatory) are at high risk (29%) of having biopsy-verified cervical intraepithelial neoplasia, and the gravity of the disparity is demonstrated by our observation that 35% of the patients with cervical intraepithelial neoplasia had lesions of greater severity than grade 1.

The insensitivity of a Papanicolaou smear to diagnose cervical intraepithelial neoplasia in the presence of histologically verified cervical intraepithelial neoplasia is evident because 92% of the repeat smears from these patients were interpreted as negative or only atypical. Although the repeat smears in patients with biopsy-verified normal results suggest a high degree of specificity of the Papanicolaou smear technique, the overall accuracy of the method for the diagnosis of cervical intraepithelial neoplasia is encumbered by the formidable false negative rate seen in patients who were found on colposcopically directed biopsy to have cervical intraepithelial neoplasia. Perhaps our data provide an explanation for the previous report of Hulka[3] in which it was reported that 7.6% of patients with cervical atypia were ultimately found to harbor frank cervical carcinoma and provide a basis for the results reported by Nyirjesy[4] in which 75% of patients with persistently atypical smears were ultimately shown to have cervical intraepithelial neoplasia or invasive carcinoma on biopsy (not colposcopically directed).

The changes of early cervical neoplasia are a progressive continuum. Melamed and Flehinger[5] suggested that the atypical smear represents the mildest form of cervical intraepithelial neoplasia, and it is only the cytologic definition of mild dysplasia that has excluded it from consideration. The data presented here, however, do not support this, inasmuch as 10% of patients with atypical smears had cervical biopsies showing moderate dysplasia (5%), severe dysplasia (3%), and carcinoma in situ (2%).

We do not know why this rather inordinate number of patients with histologically verified intraepithelial neoplasia cytologically demonstrate atypia only. The adequacy of our technique for obtaining a representative cytologic sample was documented by critical cytologic screening, and our technique has been shown to be efficacious in several previous studies.[6,7] There

may exist a subset of patients with a distinct pathologic condition that results in this unusual constellation. This question may well be answered by our current study with the use of flow cytometry to quantify deoxyribonucleic acid to permit identification of a distinct population of such cells.

Our data indicate that all patients with squamous, endocervical, or persistent inflammatory atypia are at considerable risk of harboring an underlying intraepithelial neoplasia and that colposcopic evaluation is mandatory to significantly increase the diagnostic accuracy and to help differentiate patients with cancer precursors from those with nonneoplastic changes (Fig. 1).

I am indebted to Drs. Mitchell Morris, Silviu Kohan, Edward A. Weiner, and Geraldine Young for their contribution to this study.

REFERENCES

1. Papanicolaou GN. Atlas of exfoliative cytology. Cambridge, Massachusetts: Harvard University Press, 1954.
2. Bibbo M. The abnormal pap smear. Female Patient 1983; 8:43-50.
3. Hulka BS. Cytologic and histologic outcome following an atypical cervical smear. AM J OBSTET GYNECOL 1968;101: 190-9.
4. Nyirjesy I. Atypical or suspicious cervical smears—an aggressive diagnostic approach. JAMA 1972;222:691-3.
5. Melamed MR, Flehinger BJ. Non-diagnostic squamous atypia in cervico-vaginal cytology as a risk factor for early neoplasia. Acta Cytol 1976;20:108-10.
6. Rubio CA, Kock Y, Berglund K, Thomassen P. Studies on the distribution of abnormal cells in cytologic preparations. II. Making the smear with the cotton swab applicator. Gynecol Oncol 1980;9:127-34.
7. Garite TJ, Feldman MJ. An evaluation of cytologic sampling techniques: a comparative study. Acta Cytol 1978; 22:83-5.

Magnesium sulfate and ritodrine hydrochloride: A randomized comparison

David I. Hollander, M.D., David A. Nagey, M.D., Ph.D., and Marcos J. Pupkin, M.D.
Baltimore, Maryland

The efficacy of magnesium sulfate was analyzed in relation to ritodrine hydrochloride. Patients presenting in preterm labor between 20 and 35 weeks' gestation were prospectively randomized. Tocolysis was achieved for more than 72 hours in 35 of 40 cases (88%) where magnesium sulfate was administered and 31 of 39 cases (79%) in which ritodrine hydrochloride was infused. Delay of ≥7 days was achieved in 75% and 72% of cases, respectively. The mean dosage to achieve tocolysis was 4.5 gm/hr, in the magnesium sulfate group and 210.0 μg/hr in ritodrine hydrochloride–treated patients. The mean magnesium level to achieve tocolysis was 6.60 mg/dl. Side effects in the two groups were similar in number but less alarming in the magnesium sulfate group. Magnesium sulfate was found to be easy to administer and clinically efficacious. Its tocolytic action was found to be dose dependent and drug concentrations are easily determined. On the basis of this work and data from other investigators, magnesium sulfate should be used as the first line of tocolytic therapy with ritodrine hydrochloride as its pharmacologic backup. (AM J OBSTET GYNECOL 1987;156:631-7.)

Key words: Magnesium sulfate, ritodrine hydrochloride, premature labor

Prematurity and its prevention continue to be major problems for the obstetrician and the neonatologist. In spite of the availability of several pharmacologic agents and more aggressive management approaches, the incidence of preterm labor remains at 5% to 10% and prematurity accounts for the vast majority (66% to 75%) of neonatal morbidity and mortality.[1]

It would appear that the incidence of preterm delivery may be reduced only through a program of strong patient and physician education that constantly reminds all parties involved of the possible occurrence of preterm labor and of the advantages of early diagnosis and expeditious treatment.[2] Thus tocolytic agents are not a panacea for preterm birth[3]; however, when preventive methods do fail or when early diagnosis is made,

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Maryland School of Medicine.
Presented to the Southern Perinatal Association, New Orleans, Louisiana, February 5-7, 1986.
Received for publication February 9, 1986; revised September 9, 1986; accepted September 19, 1986.
Reprint requests: David I. Hollander, M.D., Department of Obstetrics and Gynecology, St. Barnabas Medical Center, Old Short Hills Road, Livingston, NJ 07039.

one must be fully prepared to inhibit the process through proper pharmacologic intervention.

Much work has been published concerning the available tocolytic regimens.[4-10] The relative efficacies of the drugs have been demonstrated to be similar; therefore potency may not be the major consideration. Instead the incidence and severity of adverse effects may be the deciding factor. Agents with high therapeutic-to-toxic ratios would appear most appropriate. β-Sympathomimetic agents, of which ritodrine hydrochloride is the only form approved by the Food and Drug Administration, and magnesium sulfate, an agent quite familiar to obstetricians, are the drugs that have attracted the widest recent attention for tocolysis. Direct studies comparing their clinical efficacies and adverse effects are limited.[10]

This prospective study evaluates the efficacy and adverse effects of ritodrine hydrochloride and magnesium sulfate as primary tocolytic agents in a controlled, randomized fashion.

Material and methods

Patients presenting to the University of Maryland Hospital delivery room between August 1, 1984, and December 31, 1985, with preterm uterine activity were evaluated. These patients were monitored electronically for uterine contractions. If uterine contractions at a rate of at least two in 10 minutes with a duration of a minimum of 30 seconds were noted with a coexistent change in results of cervical examination, during a period of at least 1 hour, the patient was diagnosed as being in preterm labor. Primigravid patients presenting with this pattern of uterine activity and in whom the initial pelvic examination demonstrated the cervix to be at least 2 cm dilated or 80% effaced were also included in this preterm labor group. Excluded from the study were those patients in premature labor with cervical dilation >4 cm, premature rupture of the membranes, or any other maternal or fetal condition necessitating delivery. Also exluded were patients with multiple gestation and those having gestational lengths of <20 or >35 weeks. Seventy patients met those criteria and were prospectively randomized to either ritodrine hydrochloride or magnesium sulfate for intravenous tocolysis during this time period. Randomization was generated through the use of random number tables with ritodrine hydrochloride being given for odd integers and magnesium sulfate with even integers.

All patients selected for study were given an intravenous infusion of 500 ml of 5% dextrose–0.45% sodium chloride during a period of 15 to 30 minutes during an observational period when history, physical examination, and baseline laboratory (complete blood count and serum electrolytes) and electrocardiographic tests were done. If uterine activity persisted and no contraindication to tocolysis was found, drug therapy was instituted.

Patients randomized to ritodrine hydrochloride therapy (group 1) were placed on a regimen of an initial dosage of 100 μg/min given as a constant intravenous infusion. This dosage was increased by 50 μg/min every 10 to 15 minutes until contractions ceased, a maximum of 350 μg/min was attained, or unacceptable maternal side effects developed. If tocolysis was successful, the infusion was maintained for 12 hours at the lowest effective dose to maintain uterine quiescence. Patients with failure to achieve tocolysis with intravenous ritodrine hydrochloride despite maximum drug dosages or who were unable to tolerate the medication because of adverse side effects were placed on the magnesium sulfate (group 2) regimen, and the ritodrine hydrochloride infusion was halted.

Magnesium sulfate was administered as a 4 gm bolus injection during a 20-minute period followed by an infusion of 2 gm/hr. The solution used was 40 gm of magnesium sulfate in 1000 ml of 5% dextrose in water. Dosage was increased by 1 gm/hr every 30 minutes until tocolysis was achieved or a serum magnesium concentration of 6 to 8 mg/dl was obtained. Magnesium levels were determined at 2, 6, and 12 hours during the intravenous infusion as well as each time the dosage was increased. After successful tocolysis, the infusion was decreased to the lowest effective rate to maintain effective tocolysis and to maintain serum magnesium concentrations in the range of 6 to 8 mg/dl. This management was then continued until a period of 12 hours without uterine activity was achieved. If contractions persisted despite therapeutic serum magnesium concentrations or if unacceptable adverse effects were encountered, the patients were placed in the ritodrine hydrochloride protocol as described above, and magnesium sulfate was discontinued. Patients with failure of both regimens had no further attempts at tocolysis.

In all patients, vital signs were closely monitored and recorded every 15 to 30 minutes during the initial 4 hours of infusion and then hourly. Accurate measurements of intake and urinary output were also made. Patients receiving ritodrine hydrochloride had serum potassium and glucose concentration monitoring at 6 and 12 hours into the infusion while magnesium sulfate–treated patients had deep tendon reflexes monitored every hour, with serum magnesium concentrations determined as above.

Oral tocolytic agents were administered to all patients beginning 30 minutes before discontinuation of the intravenous infusion. Ritodrine hydrochloride was administered at a dosage schedule of 10 mg orally every 2 hours for 24 hours, which was then reduced to 60 to 120 mg daily in divided doses (usually 10 mg orally every 4 hours). This was titrated to maintain a maternal

Table I. Maternal characteristics* (n = 70)

Regimen	*n*	*Age (yr)*	*Parity*	*Previous preterm deliveries*	*Weeks' gestation at entry*
Ritodrine hydrochloride only	30	21.5 ± 1	2.4 ± 1.4	1.0 ± 0.6	30.3 ± 0.6
Magnesium sulfate only	31	22.5 ± 1	2.1 ± 1.1	0.9 ± 0.6	29.6 ± 0.4
Ritodrine hydrochloride failure and magnesium sulfate failure†	4	24.8 ± 0.6	2.5 ± 1.5	1.0 ± 1	30.6 ± 0.5
Ritodrine hydrochloride failure, then magnesium sulfate success	4	18.8 ± 0.8	1.5 ± 0.5	0	28.0 ± 0.4
Magnesium sulfate failure, then ritodrine hydrochloride success	1	20	2	0	31

*No intergroup comparisons were statistically significant.

†Ritodrine hydrochloride was the first-line tocolytic in two cases; magnesium sulfate was the first in the other two.

pulse response of approximately 90 to 100 bpm. Some patients in both groups were placed on a regimen of oral terbutaline sulfate instead of ritodrine hydrochloride for maintenance therapy, 5 mg orally every 4 to 6 hours. Terbutaline was more often chosen for patients without third-party health care coverage because of its lower cost. No significant differences were noted in the use of either agent. Oral therapy was maintained until 37 completed gestational weeks. If premature labor recurred before 34 weeks with oral therapy, the patient was treated with the intravenous tocolytic regimen to which she was originally randomized. The protocol previously described was then adhered to in full.

Treatment success was defined by cessation of uterine activity and delay in delivery of ≥72 hours from the onset of tocolysis. In contrast, drug failure was defined as the inability to obtain cessation of uterine activity despite maximal dosages of ritodrine hydrochloride (350 mg/min) or, in the case of magnesium sulfate, serum magnesium levels considered therapeutic (6 to 8 mg/dl). These levels were maintained for a minimum of 30 minutes before discontinuation of therapy. The diagnosis of drug failure was also made when there was progression of cervical dilation to >4 cm, spontaneous membrane rupture, or significant maternal adverse effects that necessitated discontinuation of the medication involved in the judgment of the attending obstetrician.

Comparison of drug treatments was made with respect to maternal entrance data, cervical examination data, overall success rates, mean gestational age at delivery, total number of days gained in utero, and perinatal outcome. Adverse effects on mother and neonate were also compared. Statistical analysis with the Yates correction of the χ^2 test was performed to determine statistical significance ($p < 0.05$).

Results

Seventy patients were prospectively entered into the study protocol from August 1, 1984, to December 31, 1985. Thirty-six patients were randomized (via the random number table) to receive ritodrine hydrochloride as the first tocolytic agent prescribed. In the other 34 patients magnesium sulfate was administered first. Ritodrine hydrochloride was used an additional three times (total = 39) after failure to achieve tocolysis with magnesium sulfate. In contrast, magnesium sulfate was given in six additional cases (total = 40) when ritodrine hydrochloride was judged to be a drug failure.

Patients receiving single-agent treatment in either group or in whom both agents were used consecutively demonstrated no differences with respect to maternal age, parity, gestational age, or previous history of preterm deliveries (Table I). Results of pelvic examination on admission to the study in all groups were statistically equivalent (Table II). Statistical comparisons were made with respect to first-line therapy administered.

With respect to overall success (Table III), ritodrine hydrochloride was able to delay delivery 72 hours in 79% (31/39) of the patients to whom it was administered as either a primary or secondary agent. In 72% (28/39) of the instances of use of ritodrine hydrochloride, a delay to delivery of ≥7 days was attained. The success of ritodrine hydrochloride as a primary agent used to prevent preterm delivery for 72 hours was 83% (30/36 uses). Magnesium sulfate was able to delay delivery for 72 hours in 88% (35/40) of its uses (either primary or secondary) and for a period of at least 7 days in 75% (30/40) of uses. The efficacy of magnesium sulfate as a primary tocolytic agent in delaying delivery 72 hours was 91% (31/34 uses). Differences in the two treatment regimens, although favoring magnesium sulfate, did not demonstrate statistical significance.

A breakdown of drug failures (Table IV) after primary therapy with ritodrine hydrochloride (n = 6) demonstrates that in four of these cases (67%) magnesium sulfate was successful in delaying delivery at least 72 hours. In four of the ritodrine hydrochloride failures, a maximum dosage (350 μg/min) was attained without cessation of uterine activity, while in the other two cases maternal tachycardia of >140 bpm necessitated stoppage of the medication. In the two patients

Table II. Results of cervical examination at entry and time gained in utero

Regimen	*n*	*Cervical dilatation (cm)*	*Bishop score*	*Time gained in utero (days)*
Ritodrine hydrochloride only	30	2.1 ± 0.9	4.6 ± 1.6	32.5 ± 12.3
Magnesium sulfate only	31	2.3 ± 0.9	5.2 ± 1.3	34.2 ± 12.2
Ritodrine hydrochloride failure and magnesium sulfate failure†	4	3.0 ± 0.6	6.3 ± 1.0	1.5 ± 0.7
Ritodrine hydrochloride failure then magnesium sulfate success	4	1.9 ± 4.0	4.3 ± 0.4	20.9 ± 11.8
Magnesium sulfate failure then ritodrine hydrochloride success	1	3.5	6.0	7.75

*No intergroup comparisons were statistically significant.

†Ritodrine hydrochloride was the first-line tocolytic in two cases; magnesium sulfate was first in the other two.

Table III. Drug outcome

	3-day success		*7-day success*	
	n	%	*n*	%
First-line agent				
Ritodrine hydrochloride	30/36	83	28/36	78
Magnesium sulfate	31/34	91	29/34	85
Total usage				
Ritodrine hydrochloride	31/39	79	28/39	72
Magnesium sulfate	35/40	88	30/40	75

who failed to respond to magnesium sulfate as well as ritodrine hydrochloride, one patient developed clinical and histologic evidence of abruptio placentae while the other patient developed a picture consistent with chorioamnionitis.

After drug failures with magnesium sulfate as the primary tocolytic agent, ritodrine hydrochloride was successful in delaying delivery 72 hours in only one of three cases (33%). Of the other two patients with failure to achieve tocolysis in either protocol, one was found to have evidence of abruptio placentae, and one was diagnosed subsequently as having chorioamnionitis.

Patients receiving ritodrine hydrochloride had cessation of uterine activity with a mean dosage of 210 μg/min. This level was reached in an average of 1.56 hours. Successful tocolysis with magnesium sulfate seemed dependent on reaching a serum concentration of not less than 5.6 mg/dl. The mean concentration of magnesium sulfate in the patients for whom this agent was successful was 6.6 mg/dl. Serum magnesium concentrations obtained during the infusion (Table V) demonstrate that progressive increases in the infusion rate of magnesium sulfate during a period of 3.3 hours to an infusion rate of 4.5 gm/hr were required to produce successful tocolysis. Although the time to cessation of labor was statistically shorter in the ritodrine hydrochloride group ($p < 0.05$), no cervical examination changes in the magnesium sulfate group were demonstrated during this same time period (when tocolysis was successful).

Neonatal data including birth weight, results of Dubowitz examination, and Apgar score were statistically equivalent when, as in Tables I and II, direct comparison of the two groups' successes and failures was made (Table VI). The four patients with failure of both regimens had poorer outcomes. Neonatal morbidity did not appear to be affected differently by either drug protocol.

Maternal adverse effects secondary to the medications involved, although different in presentation and severity, were comparable in number (Table VII). In two patients receiving ritodrine hydrochloride, as previously stated, maternal tachycardia necessitated discontinuation of the infusion. Similarly, metabolic changes in the patients receiving ritodrine hydrochloride were at times significant enough to suggest treatment (potassium <2.5 mEq/L). Other cardiovascular changes, although alarming to patient and staff, did not necessitate therapy. However, magnesium sulfate was found to produce increasing lethargy in two cases with diplopia as its major symptom, which required a decrease in the dosage infused to maintain serum magnesium levels in the therapeutic (6 to 8 mg/dl) range, yet without unacceptable side effects.

Comment

In this prospective randomized study, both ritodrine hydrochloride and magnesium sulfate were capable of delaying delivery at least 72 hours in 79% and 88% of their uses, respectively. Success, however, is not attained without adverse effects. Use of tocolytic agents must therefore be both judicious and cautious.

Ritodrine hydrochloride is a β-adrenergic agent with a preferential affinity for β_2-receptors. It was specifically designed and approved for use as a tocolytic agent.[4] Its mode of action is mediated through increases in adenyl cyclase, which in turn cause increases in in-

Table IV. Drug failures (n = 13)

Primary agent	*Success of secondary agent (>72 hr)*		*Reasons for discontinuation and suspected reasons for failure*
	n	%	
Ritodrine hydrochloride (n = 8)	4/6	67	Maternal tachycardia (n = 2) Maximum dosage (350 μg/min) (n = 4) Abruptio placentae (n = 1*) Occult chorioamnionitis (n = 1*)
Magnesium sulfate (n = 5)	1/3	33	Maximum dosage (6-8 mg/dl) (n = 5) Abruptio placentae (n = 1*) Chorioamnionitis (n = 1*)

*Both agents failed.

tracellular cyclic adenosine monophosphate. The effect of cyclic adenosine monophosphate on protein kinases in the cell membrane is to cause a decrease in intracellular calcium. This in turn inhibits smooth muscle contractility.

Clinical studies have favorably compared ritodrine hydrochloride with placebo as well as with other labor-inhibiting drugs. Wesselius-de Casparis et al.,[11] in an early prospective, randomized, placebo-controlled study, demonstrated the efficacy of ritodrine hydrochloride. Other work demonstrated ritodrine hydrochloride to be clinically superior to chlordiazepoxide[12] and ethanol.[13] Additionally, uncontrolled studies preceded the prospective, placebo-controlled clinical trials in this country permitting Food and Drug Administration approval.[4]

The clinical side effects reported for ritodrine hydrochloride are related to the α- and β-adrenergic effects of the drug. Maternal tachycardia, systolic blood pressure increase, and concomitant decrease in diastolic blood pressure are common effects. Chest pain or tightness, palpitations, electrocardiographic changes, and arrhythmias have been reported.[4] β-Adrenergic agents have also been implicated in the development of pulmonary edema, perhaps through augmentation of the normal pregnancy-related increases in plasma volume, fluid and salt retention, and decreases in total peripheral resistance, colloid osmotic pressure, and hematocrit. Metabolic effects associated with the usage of ritodrine hydrochloride include hyperinsulinemia, augmented glycogenolysis, and hyperglycemia. These changes may then produce increased lactate production and metabolic acidosis. In addition, potential side effects such as headaches, nervousness, nausea, and emesis as well as paralytic ileus have been reported. Despite its verified efficacy as a tocolytic agent, the high incidence of disturbing side effects has caused many to search for alternative therapies to ritodrine tocolysis.

Tocolytic properties of magnesium ions were first described in 1959 by Hall et al.,[14] who described prolongation of labor in patients treated with magnesium sulfate. The same investigators described its effects in in vitro isolated strips of uterine muscle. The mechanism of action of this ion has not been clearly elucidated but is most probably an antagonism to calcium ion binding to sarcoplasmic reticulum.

Table V. Magnesium sulfate levels

Infusion rate (gm/hr)	*Time of level (hr, mean)*	*Mean ± SD (mg/dl)*
2	1.0	4.09 ± 0.56
3	1.6	4.80 ± 0.61
4	2.7	5.74 ± 0.96
5	3.7	7.16 ± 0.32

Tocolysis achieved: Median infusion rate = 4.5 gm/hr at 3.3 hours (6.57 ± 0.80 mg/dl, mean ± SD).

Clinical studies of the efficacy of magnesium are abundant. Steer and Petrie,[15] in 1977, first published (in this country) a comparative study of magnesium sulfate and ethanol. They were able to demonstrate an improvement in successful preterm labor treatment with magnesium dependent on an early institution of therapy. Harbert et al.[16] demonstrated a reduction of uterine activity in patients with preeclampsia with serum magnesium levels of 5 to 8 mg/dl. In the preterm labor patient being treated with magnesium sulfate as the tocolytic agent of choice, Petrie[5] reported an 80% success rate in patients receiving continuous intravenous infusions of magnesium. Nochimson et al.,[6] using a similar protocol, were able to delay delivery 24 hours in 80% of patients. More recently Spisso et al.[7] as well as Elliot[8] have also established the clinical efficacy of this salt while studies directly comparing magnesium to β-adrenergic receptor agonists have shown no difference in efficacy.

The adverse effects of hypermagnesemia include impaired deep tendon reflexes, decreased muscle tone (for instance, diplopia), decreased respirations, cardiac arrythmias, and possible respiratory and cardiac arrest.

Table VI. Neonatal data* (n = 70)

	n	Birth weight (gm)	Dubowitz examination (wk)	Agpar score	
				1 min	5 min
Ritodrine hydrochloride only	30	2416 ± 696	35.3 ± 3.0	7.5 ± 1.8	8.8 ± 1.5
Magnesium sulfate only	31	2384 ± 726	35.2 ± 4.0	7.11 ± 1.9	8.4 ± 1.7
Ritodrine hydrochloride failure and magnesium sulfate failure	4	1622 ± 334	31.0 ± 1.8	5.2 ± 2.0	6.9 ± 2.0
Ritodrine hydrochloride failure then magnesium sulfate success	4	2297 ± 777	32.8 ± 3.1	7.2 ± 2.0	8.2 ± 1.7
Magnesium sulfate failure	1	1845	31	5	7

*No intergroup comparisons were statistically significant.
†Ritodrine hydrochloride was the first-line tocolytic agent in two cases; magnesium sulfate was first in the other two.

Table VII. Adverse effects

Ritodrine (n = 38)	
Nervousness	14
Palpitations	8
Tachycardia (>140 bpm)*	4
Chest pain*	2
Nausea	4
Hypokalemia (<2.5 mEq/L)	4
Hyperglycemia (>250 mg/dl)	2
Magnesium sulfate (n = 33)	
Lethargy†	15
Nausea	7
Dry mouth	7
Dizziness	3
Nystagmus	1

*Necessitating stoppage of infusion in two cases.
†Necessitating decrease of dosage.

Adverse effects may be minimized by monitoring urinary output and clinical parameters. It should be stated that significant lethargy due to the action of ionized magnesium on the neuromuscular junction and striated muscle is frequent. Nausea, emesis, chest tightness, and pulmonary edema have also been reported.[8] Hypocalcemia has been the only metabolic change noted, and cardiovascular changes including transient hypotension, peripheral vasodilatation, and increased in stroke volume have been reported.

In the present study, magnesium sulfate demonstrated comparable clinical efficacy in direct comparison with ritodrine hydrochloride. The protocol for ritodrine hydrochloride in this study is identical to that of previous work published. Our administration algorithm for magnesium sulfate differs from those previously reported.[5-8] Serum magnesium levels of 5.8 to 7.5 mg/dl were needed to achieve adequate tocolysis. The data correlate well with results by Elliot.[8] It was possible to achieve these high concentrations by increasing the infusion to a rate of between 4 and 5 gm/hr during a period of 3 to 4 hours. This would appear to be an alternative approach to that of Spisso et al.,[7] who recommend an initial infusion rate of 8.4 gm in the first hour followed by 4.8 gm in the second hour and then a continuous effusion of 2.4 gm/hr. With either regimen, it appears clear that one needs to increase the concentration of magnesium ion in the serum to adequate levels to ensure the proper action and efficacy of this tocolytic agent. Since completion of this study, we have had success with a regimen that begins with a 4 gm bolus (during a 20-minute period), followed by a rate of 4 gm/hr, with increases to 4.5 or 5 gm/hr at 30-minute intervals, if needed. That the preterm labor patient is near the zenith with respect to plasma volume as well as glomerular filtration rate suggests the need for an increased infusion rate of magnesium sulfate necessary to achieve the high serum levels required for tocolysis.

In both the ritodrine hydrochloride and magnesium sulfate groups, oral β-sympathomimetics to continue prevention of preterm delivery as suggested by Brown and Tejani[17] were used. The two groups demonstrated an equal number of recurrences (two) of preterm labor with this protocol. This relative success is in agreement with the work by Spisso et al.,[7] who demonstrated the advantages of oral β-sympathomimetic therapy (on an outpatient basis) in a group receiving parenteral magnesium sulfate. Therefore the use of intravenous magnesium sulfate to achieve short-term tocolysis followed by an oral β-adrenergic receptor agonist, whether the drug of choice be ritodrine hydrochloride or terbutaline sulfate, to minimize recurrences appears successful and warranted as is the case for the use of magnesium sulfate after intravenous β-sympathomimetics.

Adverse effects appeared with equal frequency in the two groups; however, they were more alarming in nature in the ritodrine hydrochloride group. Unlike the adverse side effects seen with magnesium sulfate, ritodrine hydrochloride effects do not appear dose related but may be a phenomenon of receptor number and activity. This further limits the predictability of its action.

The present study further demonstrated the safety and efficacy of magnesium sulfate at high infusion rates within set therapeutic levels, which are easily obtained

and measured. It is this measurability of a serum response to therapy and a close relationship between that response and therapeutic success that allow physicians an end point with which they may gauge their therapy other than by maximum allowable dosage or by the development of intolerable adverse effects. Infusions of magnesium sulfate may be titrated to a serum concentration that seems from limited experience to be equivalent in all patients. If tocolysis is not achieved at that concentration, then preliminary data suggest that ritodrine may not succeed where magnesium has failed. The data (Table IV) demonstrate that in four of five patients in whom therapy with magnesium sulfate failed to achieve tocolysis, this failure was indeed in the patients' best interest. Although numbers are limited, a similar conclusion could not be reached in ritodrine hydrochloride failures. Some caution should be exercised in case more experience reveals greater risks of magnesium sulfate tocolysis than found in this study.

The perfect answer to the prevention of preterm delivery is probably not drug therapy. No drug is effective under all conditions and side effects are frequent. However, the efficacy of magnesium sulfate and its apparent safety appear to place this agent at the forefront of those therapies available for tocolysis. On the basis of our work and the articles of Spisso et al.,[7] Elliot,[8] and Harbert et al.,[16] it appears that magnesium sulfate appears to be the first-line tocolytic agent of choice in the treatment of premature labor. Our physicians and nurses were quite receptive to the use of magnesium sulfate as the result of their familiarity with its administration and side effects albeit at lower infusion rates. The drug appears to offer a high therapeutic-to-toxic ratio with a serum concentration response that is easy to determine.

REFERENCES

1. Rush RW, Davey DA, Segall ML. The effect of preterm delivery on perinatal mortality. Br J Obstet Gynaecol 1978;85:806.
2. Herron MA, Katz M, Creasy RK. Evaluation of a preterm birth prevention program. Obstet Gynecol 1982;59:452.
3. Tejani NA, Verma UL. Effect of tocolysis on incidence of low birth weight. Obstet Gynecol 1983;61:556.
4. Merkatz I, Peter J, Barden T. Ritodrine hydrochloride: a betamimetic agent for use in preterm labor. II. Evidence of efficacy. Obstet Gynecol 1980;56:7.
5. Petrie R. Tocolysis using magnesium sulfate. Semin Perinatol 1981;5:266.
6. Nochimson D, Petrie R, Shah B, et al. Comparison of conservative and dynamic management of premature rupture of the membranes/premature labor syndrome: new approaches to delivery of infant which may minimize the need for intensive care. Clin Perinatol 1980;7:1.
7. Spisso K, Harbert G, Thiagarajah S. The use of magnesium sulfate as the primary tocolytic agent to prevent premature delivery. Am J Obstet Gynecol 1982;142:840.
8. Elliot J. Magnesium sulfate as a tocolytic agent. Am J Obstet Gynecol 1983;147:277.
9. Cotton D, Strassner H, Hill L, Schifrin B, Paul R. Comparison of magnesium sulfate, terbutaline and a placebo for inhibition of preterm labor. J Reprod Med 1984; 29:92.
10. Tchilinguirian N, Najem R, Sullivan G, Crapara F. The use of ritodrine and magnesium sulfate in the arrest of premature labor. Int J Gynaecol Obstet 1984;22:117.
11. Wesselius de Casparis A, Thiery M, Yolesian A, et al. Results of a double blind multicentre study with ritodrine in premature labor. Br Med J 1971;3:144.
12. Sivasamboo R. Premature labor. In: Baumgarten, Wesselius de Casparis A, eds. Proceedings of the international symposium of fetal risk. Baden-Baden: 1972:16-20.
13. Lauersen NH, Merkatz IR, Tejani N, et al. Inhibition of premature labor: a multicenter comparison of ritodrine and ethanol. Am J Obstet Gynecol 1977;127:837.
14. Hall D, McGauhtey H, Corey E, Thornton N. The effects of magnesium therapy on the duration of labor. Am J Obstet Gynecol 1959;78:27.
15. Steer C, Petrie R. A comparison of magnesium sulfate and alcohol for the prevention of premature labor. Am J Obstet Gynecol 1977;129:1.
16. Harbert G, Cornell G, Thornton W. Effect of toxemia therapy on uterine dynamics. Am J Obstet Gynecol 1969;105:94.
17. Brown S, Tejani N. Terbutaline sulfate in the prevention of recurrence of premature labor. Obstet Gynecol 1981; 57:22.

Treatment of acute intrapartum fetal distress by β_2-sympathomimetics

C. Mendez-Bauer, M.D., Ph.D., Afrasiab Shekarloo, M.D., Vernon Cook, M.D., and Uwe Freese, M.D.

Chicago, Illinois, Madrid, Spain, and Montevideo, Uruguay

Patients with a diagnosis of severe intrapartum fetal distress by fetal heart rate and capillary blood pH monitoring received β_2-sympathomimetics to inhibit uterine contractions (tocolysis) while the obstetric team was preparing to deliver the fetus. Fetal heart rate and acidosis significantly improved after tocolysis; these fetuses were subsequently delivered in very good metabolic and clinical condition. The favorable effect of tocolysis on fetal homeostasis is attributed to the suppression of the ischemic effect of contractions on the placental circulation. The few fetuses having an extremely compromised placental function showed no improvement in heart rate or acidosis with tocolysis and were immediately delivered. Considering the mild side effects observed, the lack of maternal complications, and the remarkable perinatal outcome obtained, we recommend using tocolysis before delivering distressed fetuses. (Am J Obstet Gynecol 1987;156:638-42.)

Key words: Fetal distress, fetal resuscitation, tocolysis, fetal treatment

Intrapartum fetal distress is, in essence, a consequence of critically diminished fetomaternal exchanges often resulting from the ischemic effect of uterine contractions on the intervillous space (and sometimes also on the umbilical circulation) acting on an already compromised maternal placental circulation.[1] The prevailing treatment of this complication is an emergency delivery and the outcome is frequently an acidotic and depressed newborn infant.

When drugs able to effectively inhibit uterine contractions (tocolysis) became available,[2] we proposed using them to treat intrapartum fetal distress.[3] The rationale for their effect is to remove the aggravating influence of uterine contractions and hence enable the improvement of the fetal metabolic condition. Encouraging results based on a few observations were reported with this method; that is, nonacidotic and vigorous newborn infants were delivered in spite of having had serious fetal distress sometime before delivery.[3]

Subsequently, favorable results[4-9] and a small incidence of maternal and fetal side effects or complications[5, 7, 10-15] were reported with the use of tocolysis to treat intrapartum fetal distress. However, this method is not currently used in the United States, perhaps because a still larger clinical experience is needed before it will be adopted. In this study we describe our experience with the use of β_2-sympathomimetics in the treatment of fetal distress in 49 well-documented patients.

From the Perinatal Unit, Department of Obstetrics and Gynecology, the University of Health Sciences, the Chicago Medical School and Cook County Hospital, Chicago, Maternidad Santa Cristina, Madrid, and the Service of Obstetrical Physiology, Montevideo.

Received for publication April 21, 1986; revised July 30, 1986; accepted September 26, 1986.

Reprint requests: Carlos Mendez-Bauer, M.D., Department of Obstetrics and Gynecology, Cook County Hospital, 1835 West Harrison St., Chicago, IL 60612.

Material and methods

The observations included in this study were obtained from three different Perinatal Centers (Department of Obstetrics, Cook County Hospital, Chicago, Illinois; Maternity Santa Cristina, Madrid, Spain, and Service of Obstetrical Physiology, Montevideo, Uruguay) and the patients were managed basically according to the same protocol. High-risk patients were routinely monitored during labor regarding fetal heart rate, uterine contractility, and fetal capillary blood pH. Additional fetal samples were obtained within 30 to 60 minutes when consistent late decelerations, a severe cord compression pattern, or sustained bradycardia was recorded. In 49 of these patients, the diagnosis of fetal distress was confirmed when an actual fetal pH <7.20 or a descent >0.10 pH unit was found. Immediately afterward and while the obstetric team was preparing to deliver the fetus, a β_2-sympathomimetic was administered to the mother. The cervical dilatation at this time ranged from 2 to 10 cm (mode 4 to 6 cm). General infection, severe preeclampsia, cardiovascular disease, and hyperthyroidism were considered contraindications to the use of β_2-sympathomimetics.

Three different tocolytic drugs were studied: ritodrine hydrochloride (Yutopar, Prepar), orciprenaline (Alupent), and terbutaline. Ritodrine and orciprenaline were administered in a continuous intravenous infusion at doses of 200 to 350 and 20 to 30 μg/min, respectively. The dose was adjusted to inhibit uterine contractions

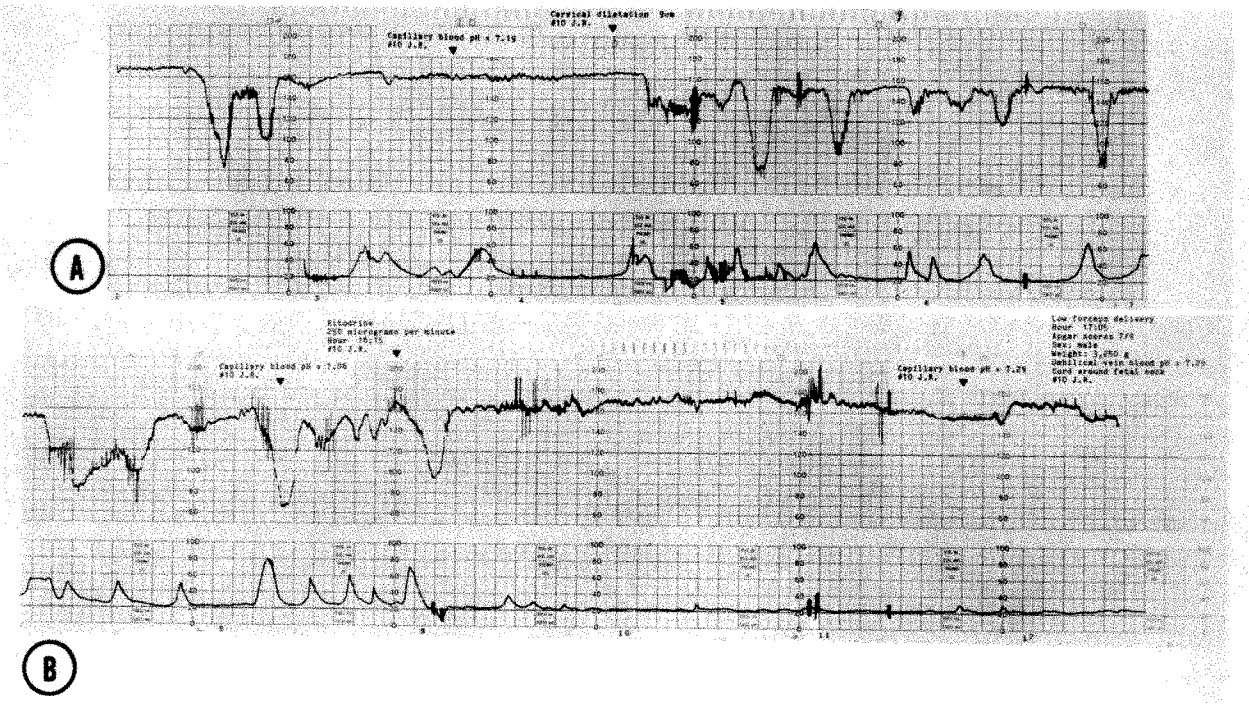


Fig. 1. Typical effect of β_2-sympathomimetics. A fetus with a capillary blood pH of 7.19 developed a severe cord compression pattern *(top)* and in 45 minutes the fetal pH fell from 7.19 *(A)* to 7.06 *(B)*. Six minutes later, after a ritodrine infusion was started, the fetal heart rate improved, after the disappearance of uterine contractions *(B)*. A fetal sample obtained 28 minutes after the onset of tocolysis shows a fetal pH of 7.29. The infant was delivered 22 minutes later by forceps. Apgar socres were 7 and 9. Umbilical vein blood pH was 7.29. The umbilical cord was wound around the fetal neck. Paper speed = 1 cm/min.

with minimal side effects. The duration of the infusion was 20 to 70 minutes (mode 20 to 30 minutes). Terbutaline was used as a single intravenous bolus injection of 0.25 mg. Twenty-five patients received ritodrine, 14 orciprenaline, and the remaining 10 terbutaline; no other therapeutic measure was used.

When the fetal heart rate showed a rapid improvement, that is, the late or variable decelerations markedly diminished in amplitude or totally disappeared or bradycardia reversed to a normal baseline level, the blood sampling was repeated within 10 to 20 minutes. If the fetal pH improved, the fetus was allowed to remain in utero until reaching a pH of $\geq$7.25 before being delivered. If no improvement occurred in the fetal heart rate or if the improvement was not accompanied by an increase in fetal pH, the fetus was immediately delivered.

Maternal age ranged from 15 to 39 years (mean = 24.0; SD = 6.4) and gestational age from 34 to 43 weeks of amenorrhea (mean = 39.8; SD = 2.12). The mean neonatal weight was 3167 gm (SD = 532.89). pH of umbilical venous blood, Apgar scores at the first and fifth minutes, neonatal body weight, maternal arterial pressure, and pulse rate were also recorded.

The data of the 37 fetuses who had successful sampling before, during, and after fetal distress were analyzed by a repeated measures analysis of variance. To determine which pairs of means were significantly different, the conditions were compared with a paired Student's *t* test.

Table I. Values of fetal blood pH (all samples included)

	pH		
	Mean	*SD*	*n*
Before distress	7.266	0.045	39
During distress	7.121	0.048	49
After β_2-sympathomimetics	7.239	0.073	44
Umbilical vein	7.256	0.072	47

Results

Except when otherwise stated, no significant differences ($p > 0.05$) were identified among the three β_2-sympathomimetic drugs used. A typical observation on the effect of tocolysis on fetal heart rate and pH is shown in Fig. 1 (*A* and *B*). The values of fetal blood pH are summarized in Table I. As fetal distress developed, the mean fetal pH decreased from 7.266 to 7.121

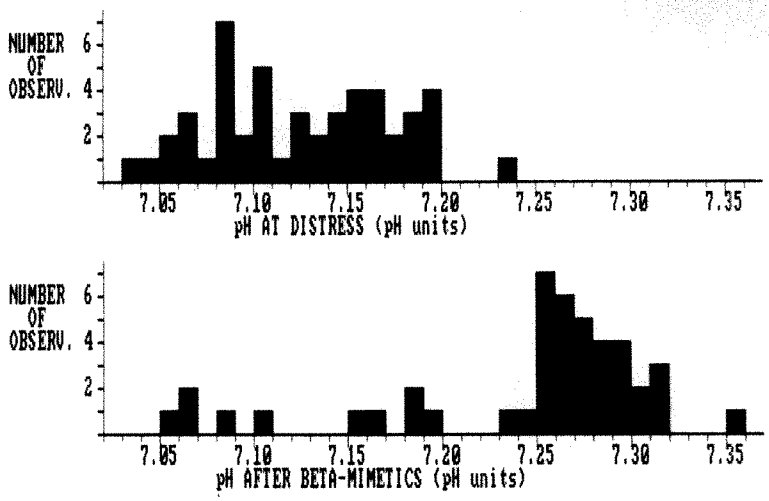


Fig. 2. *Top*: During fetal distress all fetuses but one (see text) had a pH lower than 7.20. After tocolysis *(bottom)* in 37 fetuses pH was >7.18 and in 34 it was >7.23. The modal pH after tocolysis was 7.25. All sampled cases are included. (See also Table I.)

Table II. Analysis of variance for repeated measures including only the fetuses with successful sampling before, during, and after tocolysis (F value = 87.29, $p < 0.0005$)

	pH		
	Mean	*SD*	*n*
Before distress	7.266	0.046	37
During distress	7.121	0.045	37
After tocolysis	7.242	0.071	37

and at the time of distress all fetuses had a capillary blood pH <7.20, except for one fetus with a diagnosis of fetal distress because the pH decreased from 7.34 to 7.23 in 65 minutes (see Fig. 2, *top*). After the administration of β_2-sympathomimetics, the average scalp pH rose from 7.121 to 7.239 (Table I); in 37 of the 44 fetuses sampled before delivery (84.1%) the pH was >7.18 and in 34 of them (77.3%) the pH was >7.23 (Fig. 2, *bottom*).

The statistical analysis of the pH values obtained in the 37 fetuses who were sampled before, during, and after distress (after β_2-sympathomimetics) showed overall significant differences for the three conditions (Table II). The difference between the mean values obtained before distress and after β_2-sympathomimetics was not significant, whereas the differences between the values recorded before and during distress and those during distress and after β_2-sympathomimetics were significant (Table III).

The pH values of the umbilical vein blood samples (mean = 7.256; SD = 0.072) were similar to those measured in the scalp after the administration of β_2-sympathomimetics (Table I). In 41 of them (87.2%) the pH was >7.19 and in 40 (81.1%) it was >7.24.

After administration of β_2-sympathomimetics, a marked improvement in the fetal heart rate accompanying the fetal pH increase was noticed in 34 of the 41 patients having reliable fetal heart rate tracings. In one patient (No. 21) the fetal pH remained unchanged at 7.08, although ritodrine succeeded in suppressing the uterine contractions and the fetal heart rate rose from 85 to 125 bpm.* The remaining six fetuses, who showed no definite changes either in the heart rate or in the scalp pH after the administration of β_2-sympathomimetics, were promptly delivered by cesarean section (five patients) or forceps (one patient, No. 35). The clinical complications identified in these fetuses are listed in Table IV.

The fetal acidosis (Tables I and III) developed quite rapidly (average 45.1 minutes) and after tocolysis the pH recovered even faster (average 37.1 minutes, see Table V). As mentioned above, the pH changes recorded from normal to distress and from distress to after tocolysis (Table III) were very similar.

Most patients (40) were delivered by cesarean section, under general or epidural anesthesia.† Nine patients were close to full dilatation when fetal distress developed; eight of these fetuses recovered during tocolysis and subsequently uterine contractions were allowed to reestablish. Five of them were delivered by forceps application and three had a normal spontaneous vaginal

*This 41-week-old fetus was delivered by a cesarean section 25 minutes after being diagnosed with fetal distress, with Apgar scores of 3 and 4 (at the first and fifth minute, respectively) and a body weight of 2250 gm. We have no satisfactory explanation for this unusual behavior.

†Some cesarean sections were performed with the patients under epidural anesthesia because they were already under that anesthesia during labor.

Table III. Differences in mean pH values

Time	*Difference*	*SD*	t	*p*	*n*
Before distress–during distress	0.145	0.046	19.79	<0.0005	39
Before distress–after tocolysis	0.024	0.082	1.76	0.087	37
During distress–after tocolysis	−0.118	0.085	−9.26	0.0005	44

Difference in values before distress and after tocolysis was not significant (paired Student's *t* test, $p = 0.087$). Differences in values before distress and during distress and during distress and after tocolysis were significant ($p < 0.005$).

Table IV. Clinical complications identified in fetuses who showed no definite improvement in heart rate and pH after β_2-sympathomimetics

Fetal pH		*Apgar score*		
At distress	*After β_2-sympathomimetics*	*1 min*	*5 min*	*Clinical complications*
7.06	7.12	4	6	Intrauterine growth retardation, short cord
7.10	7.10	5	5	Class B diabetes
7.15	7.12	5	6	Short cord
7.05	7.07	3	6	Abruptio placentae
7.06	7.05	3	5	Tight true knot, short cord
7.18	7.16	2	3	Multiple congenital malformations

Table V. Time elapsed (in minutes) between fetal samples obtained before and during fetal distress and during fetal distress to after tocolysis

Time	*Mean*	*SD*	*n*
Before distress–during distress	45.1	27.97	39
During distress–after tocolysis	37.1	15.3	47

Table VI. List of clinical complications probably responsible for fetal distress

Complication	*No. of patients*
Cord complication	21
Preeclampsia	9
Unknown	8
Placental lesions	6
Intrauterine growth retardation	4
Oligohydramnios	4
Diabetes	3
Postmaturity	2
Congenital malformation	2
Hypercontractility	2

delivery. All of them had Apgar scores of ≥7 at the first minute. The remaining patient (No. 35) showed no fetal improvement with tocolysis and was delivered as an emergency by forceps (Apgar scores of 3 and 5 at the first and fifth minutes).

No fetal or early neonatal losses occurred in this series and the clinical progress of these neonates was unremarkable, except for one infant who died on the tenth day of extrauterine life as a consequence of multiple congenital malformations. A low one-minute Apgar score (≤6) was found in 13 of the 49 newborn infants (26.5%), although four of these had a scalp pH >7.25 before delivery and in the umbilical vein blood. At 5 minutes of extrauterine life, 43 neonates had an Apgar score of ≥7.

The clinical complications probably responsible for the fetal distress are listed in Table VI and those identified in the six patients who showed no improvement with β_2-sympathomimetics are presented in Table IV.

Detailed maternal data on cardiovascular and neurological side effects are available only for the patients who received ritodrine or orciprenaline. At the therapeutic dose level, these patients had a significantly increased ($p < 0.005$) average heart rate, from 74.3 to 98.4 bpm. The average systolic and diastolic pressures remained almost unchanged (128 to 132 and 72.1 to 70.0 mm Hg, respectively). Well-tolerated mild tremors and nervousness were reported in 11 patients, 10 of them with orciprenaline and one with ritodrine. The average fetal heart rate increased 21.0 bpm ($p < 0.01$). Spontaneous or oxytocin-induced postpartum uterine contractility was normal in all cases.

The estimated maternal blood loss was under 500 ml in vaginal deliveries and under 1000 ml in cesarean sections. No neonatal complications were identified that could be related to the use of β_2-sympathomimetics.

Comment

The outcome of this series, in terms of umbilical blood pH and perinatal mortality and morbidity, is definitely closer to that of a normal population than to that of a group of seriously distressed fetuses. This is very intriguing when the marked degree of acidosis developed by most of these fetuses is considered (Figs. 1 and 2, Tables I, II, and III) and their rapid deterioration (Table V). Assuming a linear relationship

between the pH change from normal to distress and the time elapsed,[1, 16] the resulting average value (0.0037 pH units per minute, SD = 0.002) would classify these fetuses as having acute or even superacute fetal distress according to Saling's classification.[1, 16]

After tocolysis, most of these fetuses recovered almost as rapidly as they deteriorated (Table V), which suggests that the uterine contractions were the major distressing factor since, as soon as they disappeared, the placental function sufficed to rapidly correct fetal acidosis. We may conclude that the fetal improvement observed after tocolysis was mainly determined by the inhibition of uterine contractions, although some direct influence of these drugs on the fetomaternal homeostasis and cardiac output[5, 7, 10, 12-15] cannot be totally excluded.

In six fetuses, tocolysis failed to restore the fetal homeostasis. The kind of complication identified as the probable cause of distress in these fetuses (Table II) may explain why in utero inhibition was unable to improve fetal condition. When fetal acidosis results from a tight true knot or a short umbilical cord, an extensive abruptio placentae, or a fetal cardiovascular malformation, it will not be corrected only by suppressing uterine contractions. The main cause for fetal compromise in these cases is a very serious impairment of placental or fetal function and the ischemic effect of contractions on the maternal blood flow through the intervillous space plays a very secondary role.

With only one exception, the fetal heart rate closely reflected the improvement of fetal acidosis. A practical consequence of this finding is that when β_2-sympathomimetics are used to treat fetal distress, the evolution of fetal pH can be reasonably predicted by a careful evaluation of the fetal heart rate tracing. When the fetal heart rate bradycardia, late decelerations, or serious variable pattern fades out, a progressive recovery of fetal acidosis usually follows. These fetuses may remain "in utero," enabling the placenta to balance the altered fetal homeostasis before birth and lead to the delivery of a vigorous neonate, with an already corrected acidosis, a good cardiovascular function, and a favorable prognosis. When the fetal heart rate remains ominous, no improvement in fetal homeostasis is to be expected; these fetuses should be delivered as soon as possible. Ideally, the obstetric team should be ready to perform an emergency cesarean section when no improvement in the fetal heart rate is detected after tocolysis.

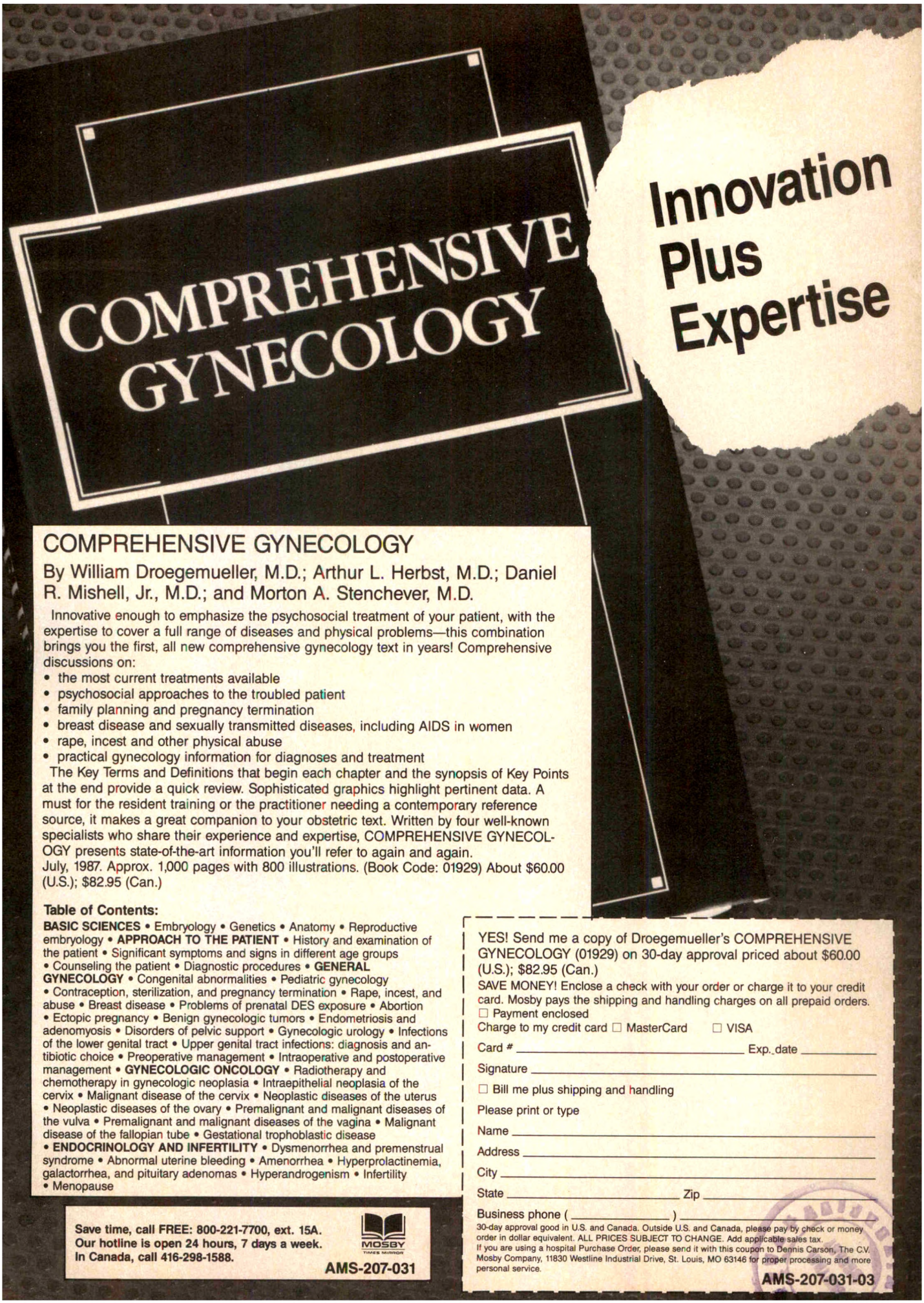
Finally, it should be noted that cesarean section was avoided in a few cases (nine vaginal deliveries, 18.36%) primarily because the fetal distress occurred when the cervix was close to full dilatation and the lapse without contractions allowed the recovery from fetal acidosis.

Considering the benefits obtained from the use of tocolysis, that is, the lack of maternal, fetal, and neonatal mortality, the reduced perinatal morbidity, and the mild side effects observed by us and other authors, we can conclude that there is enough evidence to support the use of β_2-sympathomimetics in treating acute intrapartum fetal distress if the proper technique and indications are followed.

We want to thank Dr. Marija Norusis, Associate Professor, Preventive Medicine, Rush-Presbyterian–Saint Luke's University, Chicago, Illinois, for kindly supervising the statistical analysis of the data.

REFERENCES

1. Caldeyro-Barcia R, Mendez-Bauer C, Pose SV, et al. Fetal monitoring in labor. In: Wallace HM, Gold HM, Lis E, eds. Maternal and child health practices. Springfield, Illinois: Charles C Thomas, 1973:332-94.
2. Hendricks C, Cibils LA, Pose SV, et al. The pharmacological control of excessive uterine activity with isoxsuprine. AM J OBSTET GYNECOL 1961;82:1064-77.
3. Caldeyro-Barcia R, Magana JM, Mendez-Bauer C, et al. A new approach to the treatment of acute intrapartum fetal distress. In: Proceedings of the eighth meeting of the Pan American Health Organization Committee on Medical Research. Special session. June 10, 1969. Washington DC: Pan American Health Organization, 1969: 248-53; PAHO Scientific Publication No 185.
4. Gamissans O, Esteban-Altirriba J, Calaf J. Inhibition of human myometrial activity by a new beta-adrenergic drug (DU-21220). J Obstet Gynaecol Br Commonw 1969; 75:656.
5. Lipshitz T. Use of β_2-sympathomimetic drug as a temporizing measure in the treatment of acute fetal distress. AM J OBSTET GYNECOL 1977;129:31-6.
6. Arias F. Intrauterine resuscitation with terbutaline: a method for management of acute intrapartum fetal distress. AM J OBSTET GYNECOL 1978;131:39-43.
7. Esteban-Altirriba J, Cabero L, Calaf J. Correction of fetal homeostatic disturbances. In: Aladjem S, Brown AK, Sureau C, eds. Clinical perinatology. St. Louis: CV Mosby 1980:100-12.
8. Hutchon DJR. Management of severe fetal bradycardia with ritodrine. Br J Obstet Gynaecol 1982;89:671-4.
9. Tejani M, Verma U, Chatterjee S, et al. Terbutaline in the management of acute intrapartum fetal acidosis. J Reprod Med 1983;28:857-61.
10. Bienarz J, Ivankovich A, Scommegna A. Cardiac output during ritodrine treatment in premature labor. AM J OBSTET GYNECOL 1974;118:910-20.
11. Humphrey M, Chang A, Gilbert M. The effect of intravenous ritodrine on the acid-base status of the fetus during the second stage of labor. Br J Obstet Gynaecol 1975;82:234-45.
12. Smythe AR, Sakakini J Jr. Maternal metabolic alterations secondary to terbutaline therapy for premature labor. Obstet Gynecol 1981;57:566-70.
13. Richards SR, Chang FE, Stempel LE. Hyperlactacidemia associated with acute ritodrine infusion. AM J OBSTET GYNECOL 1983;146:1-5.
14. Bassett JM, Burks AH, Levine DH, et al. Maternal and fetal metabolic effects of prolonged ritodrine infusion. Obstet Gynecol 1985;66:755-61.
15. O'Grady JP. Control of uterine activity: a clinical approach. In: Rathi M, ed. Clinical aspects of perinatal medicine. New York: Macmillan, 1985:1-27.
16. Saling E, Schneider D. Biochemical supervision of the fetus during labor. J Obstet Gynaecol Br Commonw 1967;74:799-811.



COMPREHENSIVE GYNECOLOGY

By William Droegemueller, M.D.; Arthur L. Herbst, M.D.; Daniel R. Mishell, Jr., M.D.; and Morton A. Stenchever, M.D.

Innovative enough to emphasize the psychosocial treatment of your patient, with the expertise to cover a full range of diseases and physical problems—this combination brings you the first, all new comprehensive gynecology text in years! Comprehensive discussions on:

- the most current treatments available
- psychosocial approaches to the troubled patient
- family planning and pregnancy termination
- breast disease and sexually transmitted diseases, including AIDS in women
- rape, incest and other physical abuse
- practical gynecology information for diagnoses and treatment

The Key Terms and Definitions that begin each chapter and the synopsis of Key Points at the end provide a quick review. Sophisticated graphics highlight pertinent data. A must for the resident training or the practitioner needing a contemporary reference source, it makes a great companion to your obstetric text. Written by four well-known specialists who share their experience and expertise, COMPREHENSIVE GYNECOLOGY presents state-of-the-art information you'll refer to again and again.
July, 1987. Approx. 1,000 pages with 800 illustrations. (Book Code: 01929) About $60.00 (U.S.); $82.95 (Can.)

Table of Contents:

BASIC SCIENCES • Embryology • Genetics • Anatomy • Reproductive embryology • **APPROACH TO THE PATIENT** • History and examination of the patient • Significant symptoms and signs in different age groups • Counseling the patient • Diagnostic procedures • **GENERAL GYNECOLOGY** • Congenital abnormalities • Pediatric gynecology • Contraception, sterilization, and pregnancy termination • Rape, incest, and abuse • Breast disease • Problems of prenatal DES exposure • Abortion • Ectopic pregnancy • Benign gynecologic tumors • Endometriosis and adenomyosis • Disorders of pelvic support • Gynecologic urology • Infections of the lower genital tract • Upper genital tract infections: diagnosis and antibiotic choice • Preoperative management • Intraoperative and postoperative management • **GYNECOLOGIC ONCOLOGY** • Radiotherapy and chemotherapy in gynecologic neoplasia • Intraepithelial neoplasia of the cervix • Malignant disease of the cervix • Neoplastic diseases of the uterus • Neoplastic diseases of the ovary • Premalignant and malignant diseases of the vulva • Premalignant and malignant diseases of the vagina • Malignant disease of the fallopian tube • Gestational trophoblastic disease • **ENDOCRINOLOGY AND INFERTILITY** • Dysmenorrhea and premenstrual syndrome • Abnormal uterine bleeding • Amenorrhea • Hyperprolactinemia, galactorrhea, and pituitary adenomas • Hyperandrogenism • Infertility • Menopause

Save time, call FREE: 800-221-7700, ext. 15A.
Our hotline is open 24 hours, 7 days a week.
In Canada, call 416-298-1588.

MOSBY
TIMES MIRROR

AMS-207-031

YES! Send me a copy of Droegemueller's COMPREHENSIVE GYNECOLOGY (01929) on 30-day approval priced about $60.00 (U.S.); $82.95 (Can.)

SAVE MONEY! Enclose a check with your order or charge it to your credit card. Mosby pays the shipping and handling charges on all prepaid orders.

☐ Payment enclosed

Charge to my credit card ☐ MasterCard ☐ VISA

Card # _____ Exp. date _____

Signature _____

☐ Bill me plus shipping and handling

Please print or type

Name _____

Address _____

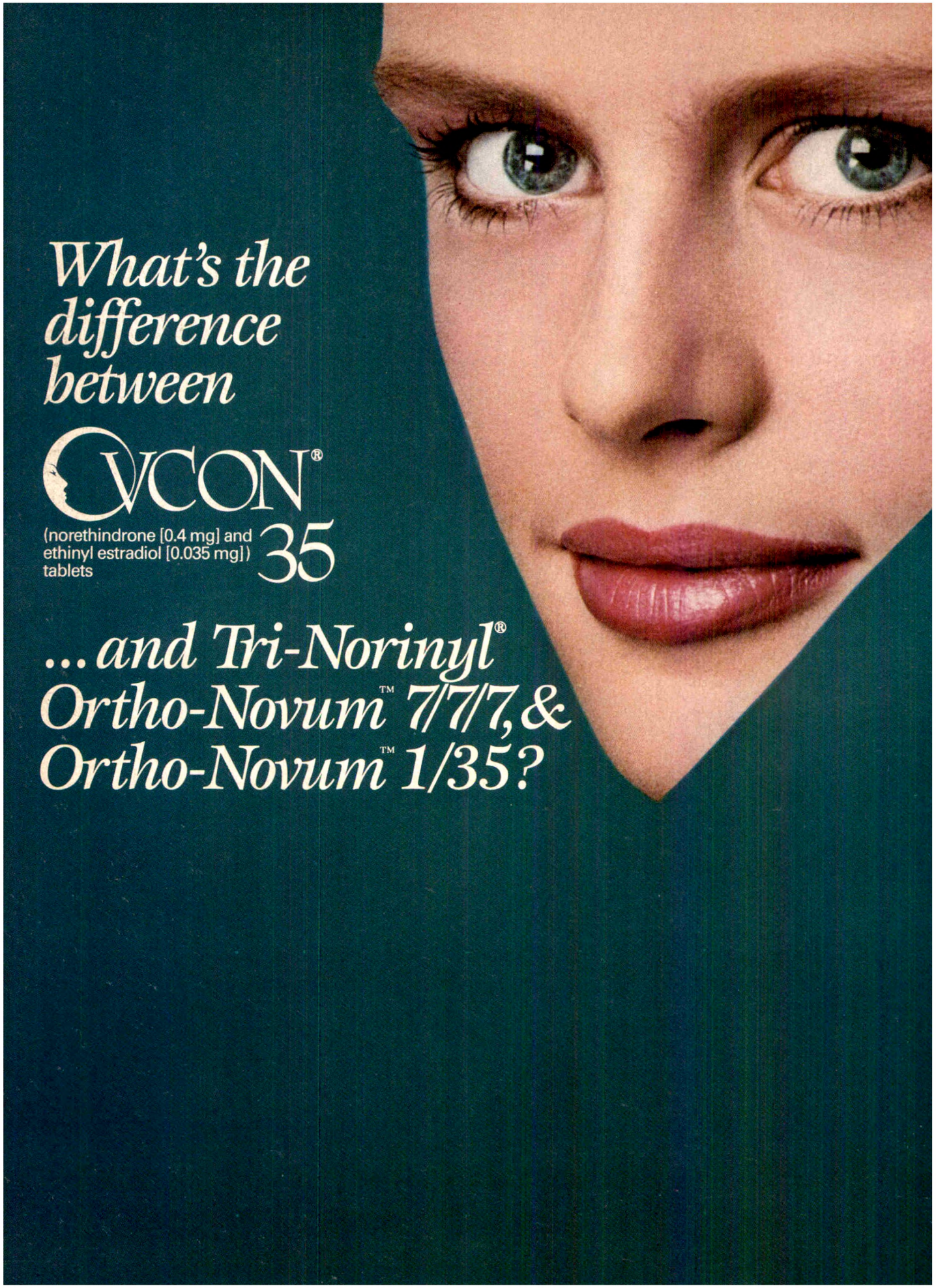
City _____

State _____ Zip _____

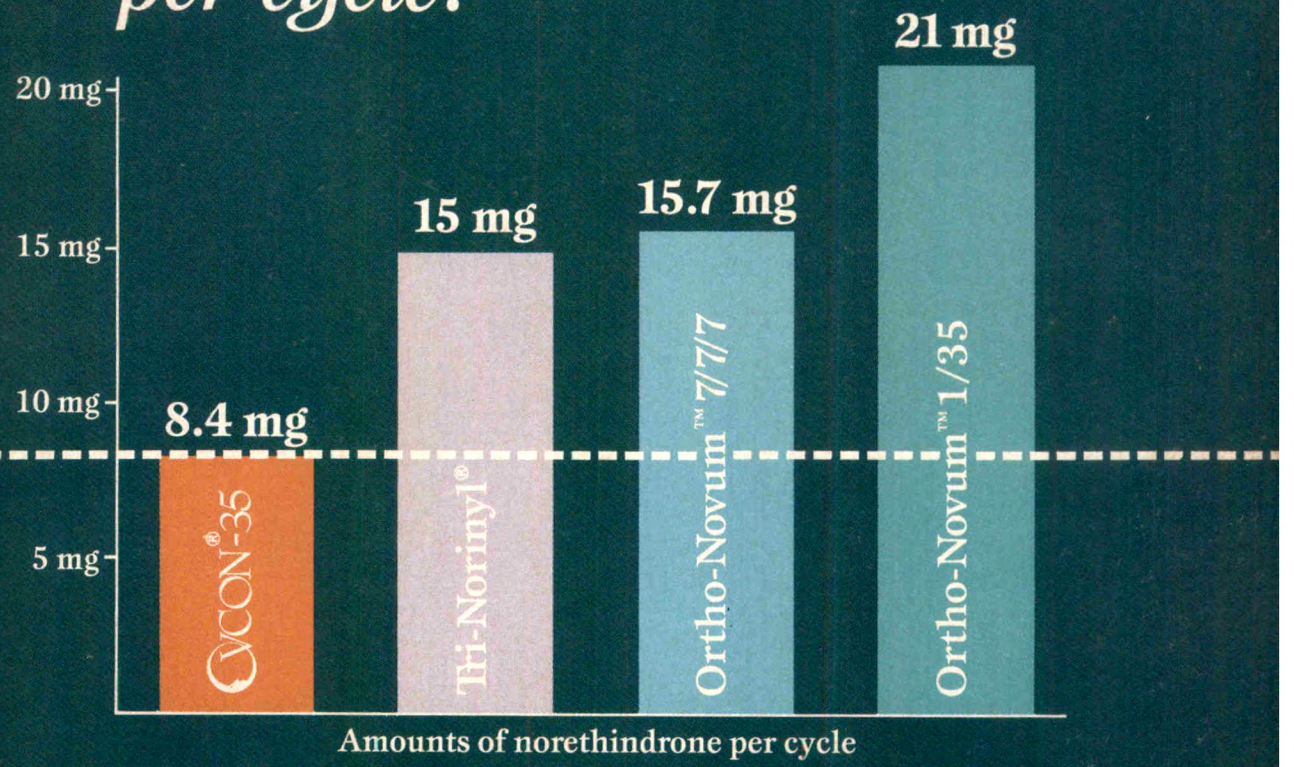
Business phone (_____) _____

30-day approval good in U.S. and Canada. Outside U.S. and Canada, please pay by check or money order in dollar equivalent. ALL PRICES SUBJECT TO CHANGE. Add applicable sales tax.
If you are using a hospital Purchase Order, please send it with this coupon to Dennis Carson, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, MO 63146 for proper processing and more personal service.

AMS-207-031-03


What's the difference between
OVCON®
(norethindrone [0.4 mg] and ethinyl estradiol [0.035 mg]) tablets
35
...and Tri-Norinyl®
Ortho-Novum™ 7/7/7,&
Ortho-Novum™ 1/35?

they contain 78% to 150% more norethindrone per cycle!



the OC with the lowest norethindrone dose

OVCON®-35

(norethindrone [0.4 mg] and ethinyl estradiol [0.035 mg]) tablets

Mead Johnson

OVCON®-50

(norethindrone [1 mg] and ethinyl estradiol [0.05 mg]) tablets

OVCON®-35

(norethindrone [0.4 mg] and ethinyl estradiol [0.035 mg]) tablets

Indications and Usage: Ovcon is indicated for the prevention of pregnancy. Some combination products containing 35 mcg. or less of ethinyl estradiol report slightly higher pregnancy rates than are reported for the higher dose combination products.

Dose-related Risk of Thromboembolism from Oral Contraceptives: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing 50 mcg. or less of estrogen.

Contraindications: Oral contraceptives should not be used in women with any of the following conditions: 1) Thrombophlebitis or thromboembolic disorders. 2) A past history of deep vein thrombophlebitis or thromboembolic disorders. 3) Cerebral vascular or coronary artery disease. 4) Known or suspected carcinoma of the breast. 5) Known or suspected estrogen dependent neoplasia. 6) Undiagnosed abnormal genital bleeding. 7) Known or suspected pregnancy. 8) Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

Warnings:

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. Women who use oral contraceptives should be strongly advised not to smoke. Use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1) *Thromboembolic Disorders and Other Vascular Problems.* An increased risk of thromboembolic and thrombotic disease associated with use of oral contraceptives is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. In a study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater. An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported. Studies conducted in the United Kingdom found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction compared to nonusers who do not smoke. Oral contraceptive users who smoke have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. The amount of smoking is a very important factor. British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives; preparations containing 100 mcg. or more of estrogen were associated with a higher risk of thromboembolism than those containing 50–80 mcg. of estrogen. The relative risk of thromboembolic disease associated with progestin-only oral contraceptives has not been determined. Cases of thromboembolic disease have been reported in women using progestin-only products, and they should not be presumed to be risk-free. The overall excess mortality rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15–34, 5/100,000; ages 35–44, 33/100,000; ages 45–49, 140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. The highest risk was found in heavy cigarette smokers (15 or more cigarettes per day) who used oral contraceptives and were aged 40 or older. Women who smoke should be advised not to use oral contraceptives. The use of oral contraceptives in women over age 40 with other risk factors is not recommended. The mortality associated with all of the methods of birth control is low compared to the risk of childbirth, with the exception of oral contraceptive users who smoke and are over age 40. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and for myocardial infarction is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia, and especially by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. If feasible, oral contraceptives should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2) *Ocular Lesions.* There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue the medication if there is unexplained sudden or gradual, partial or complete loss of vision; sudden onset of proptosis or diplopia; papilledema; or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3) *Carcinoma.* Long term administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. In humans, one study investigated the first 21 cases of endometrial adenocarcinoma in women on oral contraceptives reported to a registry. Of those women without predisposing risk factors for this disease, nearly all occurred in women who had used a sequential oral contraceptive. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestin-only oral contraceptives. No increase in breast cancer in women taking oral contraceptives has been reported although one study reported an increased risk of breast cancer in subgroups of women treated using oral contraceptives with documented benign breast disease. There is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives. 4) *Hepatic Adenoma.* Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study reported a higher risk associated with oral contraceptive formulations with high hormonal potency. Although benign and rare, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short term as well as long term users of oral contraceptives. Two studies relate the risk with duration of contraceptive use, the risk being much greater after 4 or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5) *Use in Pregnancy; Birth Defects in Offspring and Malignancy in Female Offspring.* Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. A high percentage of such exposed women (30% to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Similar data are not available with the use of other estrogens but it cannot be presumed that they would not induce similar changes. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000 live births. In the past, female there is no evidence from well-controlled studies that progestins are effective for these uses. Increases in chromosomal aberrations have been reported in women who become pregnant soon after ceasing oral contraceptive therapy. Embryos with these anomalies are virtually always spontaneously aborted. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed the patient should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation should be discussed in light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months. Administration of progestin-only or progestin-estrogen combinations to induce withdrawal bleeding should not be used as a test for pregnancy. 6) *Gall Bladder Disease.* Studies have reported an increased risk of surgically confirmed gallbladder disease appearing after one year of oral contraceptive use and doubling of the risk after 4 or 5 years of use. 7) *Carbohydrate and Lipid Metabolic Effects.* A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. Increased serum levels of triglycerides and total phospholipids have been observed in oral contraceptive users. The clinical significance of this observation is unknown at this time. 8) *Elevated Blood Pressure.* An increase in blood pressure has been reported in women receiving oral contraceptives. The prevalence of hypertension in oral contraceptive users may be no higher than nonusers in the first year of oral contraceptive use but increases with longer exposure and in the fifth year of use is two and one-half to three times the reported prevalence in the first year. Women who previously had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. 9) *Headaches.* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of oral contraceptives and investigation of the cause. 10) *Bleeding Irregularities.* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. 11) *Ectopic Pregnancy.* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in oral contraceptive failures, the ratio of ectopic to intrauterine pregnancies is higher than in women not using oral contraceptives since the drugs are more effective in preventing intrauterine rather than ectopic pregnancy. The higher ectopic-intrauterine ratio has been reported with both combination products and progestin-only oral contraceptives. 12) *Breast Feeding.* A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

Precautions: 1) A complete medical and family history should be taken prior to the initiation of oral contraceptives. Examination should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than 1 year without another physical examination being performed. 2) Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3) Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 4) Because oral contraceptives may cause some degree of fluid retention, conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, cardiac or renal insufficiency or asthma require careful observation. 5) Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6) Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7) Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8) Serum folate levels may be depressed by oral contraceptive therapy. This may complicate subsequent pregnancy with regard to folate deficiency. 9) The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10) Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T_4 by column, or T_4 by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. 11) The active yellow tablets and the inert green tablets in Ovcon-50 (21 and 28 day regimens) and the inert green tablets in the 28 day regimen of Ovcon-35 contain FD&C Yellow No. 5 (tartrazine) which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals. Although the overall incidence of FD&C Yellow No. 5 (tartrazine) sensitivity in the general population is low, it is frequently seen in patients who also have aspirin hypersensitivity.

Information for the Patient: Detailed Patient Labeling has been prepared for use by the patient and has been made available for distribution by the pharmacist.

Drug Interactions: Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Adverse Reactions: An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **Warnings**): Thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis; benign hepatomas, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle (other reactions, as a general rule, are seen much less frequently or only occasionally); gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes (tenderness, enlargement, and secretion); change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; changes in corneal curvature (steepening), intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

Acute Overdosage: Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

03

Mead Johnson LABORATORIES

C-V21
MJL 6-4184

FACULTY OF MEDECINE UNIVERSITY OF MONTREAL

The Department of obstetrics-gynecology invites application for a faculty position in perinatalogy at the Pediatric Research Center of Ste-Justine Hospital to be filled from June 1st, 1987. Candidates should hold a M.D., be certified specialist in obstetrics-gynecology and have a two-year minimum research training. A good working knowledge of the French language is required. Curriculum viate with description of research interests should be submitted by April 15, 1987 to:

Dr. Yves Lefebvre
Chairman
Dept. of obstetrics-gynecology
Faculty of Medicine
University of Montreal
P.O. Box 6128, Station A
Montreal (QUEBEC) H3C 3J7

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents.

SEATTLE GROUP PRACTICE

Fast growing women's OB/GYN Group practice

Associated with a major Seattle medical center. First year income guarantee. Malpractice and benefits provided. Practice structured to support personal as well as professional goals. High-quality medical community. New delivery facilities, strong Family Practice Residency and Department.

For information:

The Friedrich Group, Inc.
9284 Ferncliff Northeast
Bainbridge Island, WA 98110
(206) 547-7850
(206) 842-5248

FACULTY POSITION

The Knoxville Unit of The University of Tennessee College of Medicine is currently accepting applications for general specialists in the Department of Obstetrics and Gynecology at The University of Tennessee Medical Center at Knoxville.

This position requires board certification or eligibility in Obstetrics and Gynecology. Responsibilities include: resident and medical student education; participation in Regionalization Program; and clinical or basic research.

Specialized areas of interest such as: adolescent GYN; GYN urology; benign GYN surgery; and/or reproductive immunology are encouraged.

Academic rank is commensurate with qualifications and experience.

Interested persons should send curriculum vitae to:

Chairman, Department of Obstetrics and Gynecology
University of Tennessee Medical Center
1924 Alcoa Highway
Knoxville, TN 37920
(615) 544-9306

UT

An Affirmative Action/Title IX/Section 504 Employer

OBSTETRICIAN GYNECOLOGIST

Position available with The Permanente Medical Group, Inc., San Jose, California. Sixteen member OB/GYN Department seeking Board Certified/Eligible physicians for immediate openings. Excellent professional environment, competitive salary, outstanding benefits. Located in the San Francisco Bay Area, with superb educational, cultural and recreational activities.

Contact:

Jerome L. Nehemiah, M.D., Chief
Department of OB/GYN
The Permanente Medical Group, Inc.
260 International Circle
San Jose, CA 95119

The competition is significant gaps in

fact:
Excellent Clinical Results in Intra-abdominal Infections* Compared with Mefoxin® (cefoxitin/MSD)

Ref.	Investigator	Year	Therapy	Satisfactory Response (No. of cases)
1	Lou et al.	1985	Cefizox* (ceftizoxime)	100% (48/48)
			cefoxitin	98% (60/61)

Clinical response was based upon subjective evaluation by the investigator; no differentiation between cure and improvement was made.

fact:
Enteric Microflora Profile Superior to Mefoxin®

Enteric Pathogens†		Cefizox®	Mefoxin®
Refs.	Organisms (No. of strains)	MIC_{90} (mcg./ml.)	MIC_{90} (mcg./ml.)
2	*Escherichia coli (40)*	0.04	6.3
2	*Klebsiella pneumoniae (32)*	0.05	25
2	*Enterobacter aerogenes (16)*	0.12	>400
2	*Proteus* (indole+) *(26)*	6.3	12.5
2	*Serratia marcescens (32)*	12.5	>100
3	*Providencia rettgeri*	0.06	64
4	*Bacteroides fragilis (55)*	8	16

*Due to susceptible strains of indicated organisms.
†*In vitro* activity does not necessarily imply *in vivo* effectiveness.

1. Lou, M.A., et al.: Comparative evaluation of ceftizoxime and cefoxitin in the treatment of intraabdominal infections, 14th Internat. Congr. Chemother., June 1985, Kyoto, Japan, pp. 2378-2379.
2. Fu, K.P., and Neu, H.C.: Antibacterial activity of ceftizoxime, a β-lactamase-stable cephalosporin, *Antimicrob. Agents Chemother.* 17:583-590, Apr. 1980.
3. Thornsberry, C.: Review of in vitro activity of third-generation cephalosporins and other newer beta-lactam antibiotics against clinically important bacteria, *Am. J. Med.* 79: (Suppl. A) 14-20, Aug. 1985.
4. Aldridge, K.E., et al.: Comparison of the activities of penicillin G and new β-lactam antibiotics against clinical isolates of *Bacteroides* species, *Antimicrob. Agents Chemother.* 26:410-413, Sept. 1984.

fact:
Lower Cost of Therapy than Mefoxin®

Average Hospital Costs of Therapy for Severe Infections	Cefizox®	Mefoxin®
Cost per Unit	$9.13/g.*	$6.82/g.
Recommended Dosage for Severe Infections	2g. q8-12h	2g. q6-8h
Cost per Day†	$55 to $37	$55 to $41
Preparation and Administration Cost per Day‡	$9 to $6	$12 to $9
Total Cost of Therapy per Day	$64 to $43	$67 to $50
Total Cost of Therapy per 7 Days of Treatment	$448 to $301	$469 to $350
Savings with CEFIZOX®	**$21 to $49**	

* Price quoted = wholesaler's price + 5%.
† Rounded to the nearest dollar.
‡ Estimated @ $3/dose.

- **Offers b.i.d. dosing for all but life-threatening infections**
- **Outstanding mixed aerobic/anaerobic coverage**
- **Excellent safety profile**
- **Lower cost of therapy than Mefoxin®**

right! Why risk coverage?

the facts *actually* favor

Cefizox® IM IV

brand of sterile

ceftizoxime sodium

FSK

Manufactured for
Fujisawa SmithKline
Distributed by
SK&F Laboratories
Division of SmithKline Beckman Corp.

Please see brief summary of prescribing information on last page of this advertisement.

Cefizox®

brand of

sterile ceftizoxime sodium and ceftizoxime sodium injection

Before prescribing, see complete prescribing information in SK&F literature or *PDR*. The following is a brief summary.

INDICATIONS

'Cefizox' is indicated in the treatment of infections due to susceptible strains of the microorganisms listed below:

LOWER RESPIRATORY TRACT INFECTIONS caused by *Streptococcus* sp. including *S. pneumoniae* (formerly *D. pneumoniae*), but excluding enterococci; *Klebsiella* sp.; *Proteus mirabilis; Escherichia coli; Haemophilus influenzae* including ampicillin-resistant strains; *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Serratia* sp.; *Enterobacter* sp.; and *Bacteroides* sp.

URINARY TRACT INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Escherichia coli; Pseudomonas* sp. including *Ps. aeruginosa; Proteus mirabilis; P. vulgaris; Providencia rettgeri* (formerly *Proteus rettgeri*) and *Morganella morganii* (formerly *Proteus morganii*); *Klebsiella* sp.; *Serratia* sp. including *S. marcescens; Enterobacter* sp.

GONORRHEA. Uncomplicated cervical and urethral gonorrhea caused by *Neisseria gonorrhoeae.*

INTRA-ABDOMINAL INFECTIONS caused by *Escherichia coli; Staphylococcus epidermidis; Streptococcus* sp. (excluding enterococci); *Enterobacter* sp.; *Klebsiella* sp.; *Bacteroides* sp. including *B. fragilis;* and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

SEPTICEMIA caused by *Streptococcus* sp. including *S. pneumoniae* (formerly *D. pneumoniae*), but excluding enterococci; *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Escherichia coli; Bacteroides* sp. including *B. fragilis; Klebsiella* sp.; and *Serratia* sp.

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Staphylococcus epidermidis; Escherichia coli; Klebsiella* sp.; *Streptococcus* sp. including *Streptococcus pyogenes* (group A beta-hemolytic), but excluding enterococci; *Proteus mirabilis; Serratia* sp.; *Enterobacter* sp.; *Bacteroides* sp. including *B. fragilis;* and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

BONE AND JOINT INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Streptococcus* sp. (excluding enterococci); *Proteus mirabilis; Bacteroides* sp.; and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

MENINGITIS caused by *Haemophilus influenzae*. 'Cefizox' has also been used successfully in the treatment of a limited number of pediatric and adult cases of meningitis caused by *Streptococcus pneumoniae.*

'Cefizox' has been effective in the treatment of seriously ill, compromised patients, including those who were debilitated, immunosuppressed or neutropenic.

Many infections caused by aerobic gram-negative organisms and by mixtures of organisms resistant to other cephalosporins, aminoglycosides, or penicillins respond to treatment with 'Cefizox'.

Because of the serious nature of some urinary tract infections due to *Ps. aeruginosa* and because many strains of *Pseudomonas* species are only moderately susceptible to 'Cefizox', higher dosage is recommended. Other therapy should be instituted if the response is not prompt.

Susceptibility studies on specimens obtained prior to therapy should be used to determine the response of causative organisms to 'Cefizox'. Therapy with 'Cefizox' may be initiated pending results of the studies; however, treatment should be adjusted according to study findings. In serious infections 'Cefizox' has been used concomitantly with aminoglycosides (see Precautions). Before using 'Cefizox' concomitantly with other antibiotics, the prescribing information for those agents should be reviewed for contraindications, warnings, precautions and adverse reactions. Renal function should be carefully monitored.

CONTRAINDICATIONS

'Cefizox' is contraindicated in persons who have shown hypersensitivity to cephalosporin antibiotics.

WARNINGS

BEFORE THERAPY WITH 'CEFIZOX' IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE WHETHER THE PATIENT HAS HAD PREVIOUS HYPERSENSITIVITY REACTIONS TO CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. THIS PRODUCT SHOULD BE GIVEN CAUTIOUSLY TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

Pseudomembranous colitis **has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who developed diarrhea in association with antibiotic use.** Treatment with broad-spectrum antibiotics alters normal flora of the colon and may permit overgrowth of *Clostridia*. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin *in vitro*. Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C. difficile*. Other causes of colitis should also be considered.

PRECAUTIONS

General: As with all broad-spectrum antibiotics, 'Cefizox' should be prescribed with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Although 'Cefizox' has not been shown to produce an alteration in renal function, renal status should be evaluated, especially in seriously ill patients receiving maximum dose therapy. As with any antibiotic, prolonged use may result in overgrowth of nonsusceptible organisms. Careful observation is essential; appropriate measures should be taken if superinfection occurs.

Drug Interactions: Although the occurrence has not been reported with 'Cefizox,' nephrotoxicity has been reported following concomitant administration of other cephalosporins and aminoglycosides.

Pregnancy: (Category B.) Reproduction studies have been performed in rats and rabbits and have revealed no evidence of impaired fertility or harm to the fetus due to 'Cefizox'. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Labor and Delivery: Safety of 'Cefizox' use during labor and delivery has not been established.

Nursing Mothers: 'Cefizox' is excreted in human milk in low concentrations. Caution should be exercised when 'Cefizox' is administered to a nursing woman.

Infants and Children: Safety and efficacy in infants from birth to six months of age have not been established. In children six months of age and older, treatment with 'Cefizox' has been associated with transient elevated levels of eosinophils, SGOT, SGPT and CPK (creatine phosphokinase). The CPK elevation may be related to I.M. administration.

ADVERSE REACTIONS

Ceftizoxime is generally well tolerated; the *most* frequent adverse reactions (*greater than* 1%, but less than 5%) are: *Hypersensitivity*—Rash, pruritus, fever. *Hepatic*—Transient elevation in SGOT, SGPT and alkaline phosphatase. *Hematologic*—Transient eosinophilia, thrombocytosis. Some individuals have developed a positive Coombs test. *Local*—Injection site—Burning, cellulitis, phlebitis with I.V. administration, pain, induration, tenderness, paresthesia.

Less frequent adverse reactions (*less than* 1%) are: *Hypersensitivity*—Numbness has been reported rarely. *Hepatic*—Elevation of bilirubin has been reported rarely. *Renal*—Transient elevations of BUN and creatinine have been occasionally observed with ceftizoxime. *Hematologic*—Anemia, leukopenia, neutropenia and thrombocytopenia have been reported rarely. *Genitourinary*—Vaginitis has rarely occurred. *Gastrointestinal*—Diarrhea; nausea and vomiting have been reported occasionally. Symptoms of pseudomembranous colitis can appear during or after antibiotic treatment. (See Warnings.)

HOW SUPPLIED

Cefizox (sterile ceftizoxime sodium, FSK) is supplied in vials equivalent to 1 gram or 2 grams of ceftizoxime; in "Piggyback" Vials for I.V. admixture equivalent to 1 gram or 2 grams of ceftizoxime.

Cefizox (ceftizoxime sodium injection, FSK) as a frozen, sterile, nonpyrogenic solution in plastic containers—supplied in 50 ml. single-dose containers equivalent to 1 gram or 2 grams of ceftizoxime, in 5% Dextrose (D5W). Do not store above -20°C.

'Cefizox' supplied as a frozen, sterile, nonpyrogenic solution in plastic containers is manufactured for Fujisawa SmithKline Corporation by Travenol Laboratories, Inc., Deerfield, IL 60015.

BRS — CF:L14

© Smith Kline &French Laboratories, 1987
Division of SmithKline Beckman Corporation

FSK

Manufactured for
Fujisawa SmithKline Corporation
Distributed by
SK&F Laboratories
Division of SmithKline Beckman Corp.

Upper airway obstruction as a cause of pulmonary edema during late pregnancy

Urs Lagler, M.D., and Erich Russi, M.D.
Zurich, Switzerland

We describe a 35-year-old woman who developed pulmonary edema at 38 weeks of pregnancy as a result of upper airway obstruction caused by a progressively enlarging euthyroid goiter. (AM J OBSTET GYNECOL 1987;156:643-4.)

Key words: Pulmonary edema, pregnancy, upper airways obstruction, goiter

Pulmonary edema in pregnant women without preexisting heart disease may be caused by the use of β-mimetic agents for preterm labor, by severe hypertension of preeclampsia, by amniotic fluid embolism, or by septic events during delivery.

Case report

A 35-year-old Albanian woman at 38 weeks of gestation was admitted in a semicomatose, cyanotic state with extreme shortness of breath with an inspiratory stridor.

A mild euthyroid goiter had been present for 2 years. During her pregnancy, which otherwise was normal, she noticed a progressive enlargement of her thyroid gland together with dysphagia and posture-dependent dyspnea. She was finally unable to sleep unless in a sitting position. There was no history of preeclampsia, high blood pressure, or congestive heart failure.

On admission the patient was immediately intubated without difficulty with a 7.0 mm diameter endotracheal tube, and copious amounts of white foamy secretions were suctioned from the airways. Chest radiographic studies confirmed the clinical diagnosis of severe pulmonary edema. Blood pressure was 80/60 mm Hg; analysis of arterial blood gas, drawn immediately after intubation and during ventilation with an $F_{I_{O_2}}$ of 1.0, revealed a P_{O_2} of 54 mm Hg and a pH of 7.30. The pulmonary edema cleared within hours. Since the child's heart rate pattern deteriorated, a cesarean section was performed and a viable boy was delivered. The postoperative course was without complications, and the patient remained stable and well oxygenated on the ventilator. Blood pressure was 120/80 mm Hg and the pulse rate 80 per minute and regular. A cardiac ultrasound scan revealed normal chamber diameters with good contractility and no sign of mitral stenosis.

After 24 hours the patient was extubated; 45 minutes later she became severely cyanotic, tachypneic, and short of breath and developed a massive inspiratory stridor. Again an endotracheal tube was inserted without difficulties. Flexible fiberoptic bronchoscopy revealed mild laryngeal edema and a normal tracheal diameter distal to the tip of the endotracheal tube. By retracting the tube we found a 70% slit-form tracheal stenosis 7 cm above the carina caused by extrinsic compression. No malignant cells were found by fine needle aspiration of the goiter. The trachea was decompressed by subtotal strumectomy: no tracheomalacia was present. The histologic examination revealed a macrofollicular goiter. The postoperative course was uneventful.

Comment

We believe that pulmonary edema in this patient was caused by severe upper airway obstruction. The absence of a history of toxemia, arterial hypertension, left ventricular dysfunction, or valvular heart disease makes other causes of pulmonary edema in late pregnancy unlikely. Furthermore, the patient was not receiving β-mimetics and there was no evidence of amniotic fluid embolism. A strikingly similar case was reported by Stradling and Bolton.[1] Pulmonary edema caused by upper airway obstruction has been reported secondary to tumor, strangulation, and interrupted hanging and in children with croup and epiglottitis; it can be induced experimentally in dogs by increasing the inspiratory resistance.[2]

A normal pregnancy in a healthy woman does not result in pulmonary edema but may create a fragile pulmonary water balance. Blood volume is expanded: plasma volume increases by 50% in normal primagravid women and red cell mass by 18% to 30%. Furthermore, the colloid-oncotic pressure of plasma falls by about 7 cm of water. Thus it can be speculated that the fluid shift from the pulmonary capillaries to the

From the Department of Internal Medicine, University Hospital of Zurich.
Received for publication June 19, 1986; accepted September 26, 1986.
Reprint requests: E. Russi, M.D., Department of Internal Medicine, University Hospital of Zurich, Rämistrasse 100, CH-8091 Zurich, Switzerland.

alveolar space caused by complex intrathoracic pressure changes resulting from repeated Mueller maneuvers may be facilitated by the expanded intravascular volume during late pregnancy.

REFERENCES

1. Stradling JR, Bolton P. Upper airways obstruction as cause of pulmonary oedema. Lancet 1982;2:1353-4.
2. Oswalt CE, Gates GA, Holmstrom FM. Pulmonary edema as a complication of acute airway obstruction. JAMA 1977;238:1833-5.

Symphysis-fundus height, maternal height, labor pattern, and mode of delivery

Alan B. Hughes, M.D., D. Adam Jenkins, M.B., B.S., B.Sc., Robert G. Newcombe, Ph.D., and James F. Pearson, M.D.

Cardiff, Wales

Fetal growth was monitored with use of symphysis-fundus charts in 920 consecutive term singleton pregnancies with cephalic presentation. There were 402 primigravid and 518 multigravid patients. The symphysis-fundus charts were divided into four zones and the patients allocated into groups according to the zone of the last symphysis-fundus height measurement before the onset of labor. A highly significant increase was noted in the incidence of abnormal labor and operative delivery in group A primigravid women (symphysis-fundus height above the ninetieth centile), which was even more pronounced when the effect of epidural analgesia was excluded. Conversely, there was a significant progressive increase in the proportion of operative deliveries for fetal distress from group A through group D (symphysis-fundus height below the tenth centile). Similar trends were significant but less pronounced among multigravid women. The relationship between maternal height, symphysis-fundus height, and operative delivery for dystocia in primigravid women was examined. High operative delivery rates were present in group A primigravid patients regardless of maternal height. In groups B and C, however, increased maternal height was associated with lower operative delivery rates. (Am J Obstet Gynecol 1987;156:644-8.)

Key words: Symphysis-fundus height, maternal height, labor pattern, mode of delivery

Currently there is a resurgence of interest in low-cost clinical methods of measuring fetal growth. One such method that has achieved acceptance is the symphysis-fundus height chart.[1-4] It seemed to us that these charts could be further used to provide information of prognostic value during labor. For instance, one might expect the large infant to be at greater hazard from dystocia than from asphyxia, and conversely, the small infant to be at greater risk from asphyxia than from dystocia. Since fetal growth is reflected by symphysis-fundus height, reference to the pattern of symphysis-fundus growth might direct the attention of the obstetrician toward certain problems more likely to be encountered in labor. The present investigation attempts to examine this postulate and quantify this relationship.

From the Departments of Obstetrics and Gynaecology and Medical Statistics, University of Wales College of Medicine.
Received for publication May 20, 1986; revised September 18, 1986; accepted September 24, 1986.
Reprint requests: A. B. Hughes, M.D., Department of Obstetrics and Gynaecology, University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN Wales.

Patients and methods

Nine hundred twenty consecutive term singleton pregnancies with cephalic presentation were analyzed according to labor pattern and mode of delivery. There were 402 primigravid and 518 multigravid patients.

Fetal growth was monitored with use of the symphysis-fundus charts of Calvert et al.[4] (Fig. 1). Measurements were made by lightly marking the position of the uterine fundus while the patient was supine. The end of a nonelastic tape measure was held against the fixed point (symphysis) and the distance to the mark on the patient's skin recorded. Whole digit preference was decreased by use of a tape marked in inches on one side and centimeters on the other. The measurement was initially made in inches, then the tape turned

over, and the corresponding metric value recorded to the nearest half centimeter. This method has a low intraobserver coefficient of variation (4.6%) and a low interobserver coefficient of variation (6.4%). The symphysis-fundus chart was divided into four zones (Fig. 1): zone A (symphysis-fundus height above the ninetieth centile line), zone B (between the fiftieth and ninetieth centile lines), zone C (between the tenth and fiftieth centile lines), and zone D (below the tenth centile line). Patients were grouped according to the zone of the last symphysis-fundus height measurement before the onset of labor.

Gestational age was confirmed by ultrasound in early pregnancy in every case. During labor the use of continuous electronic fetal heart rate monitoring and of composite partograms as described by Studd et al.[5] was routine. Continuous lumbar epidural analgesia was provided when clinically indicated or requested by the patient and used in 27% of the primigravid and 6% of the multigravid patients. Oxytocin augmentation of labor was provided for 23% of the primigravid and 4% of the multigravid patients.

The labor pattern was considered to be normal if the rate of cervical dilatation during the active phase was at least 1.2 cm/hr in primigravid women and 1.5 cm/hr in multigravid ones.[6]

We have used the term "active-phase dystocia" to include protracted active-phase dilatation (primary dysfunctional labor), secondary arrest, and prolonged deceleration phase. The duration of the latent phase of labor was excluded from analysis. "Second-stage delay" was interpreted as having occurred when operative intervention was required for "failure to progress." This was usually compatible with Friedman's limits of 1.1 hours for multigravid patients. However, in the United Kingdom, the second stage of labor has been regarded as a time of particular risk for progressive fetal acidosis.[7, 8] Consequently for primigravid patients a time limit of 1½ hours was usually recommended before intervention with assisted delivery.

The primigravid population was subdivided according to height. "Short" stature was taken as a height of ≤156 cm, as defined by Baird,[9] and "tall" stature was defined as a height of >168 cm, so that the proportion of tall women (19%) approximated that of the short women (21%). The intermediate group (156 to 168 cm) comprised 60% of the population.

Statistical analysis. Qualitative variables were analyzed by χ^2 tests. Groups A, B, and C were compared initially by a χ^2 test on 2 degrees of freedom (df), or 4 df in the case of labor pattern or mode of delivery. For many variables group A was dissimilar to group B but group B was similar to group C: in such instances the subsidiary analysis comparing group A with group B is quoted. Although all group D results are

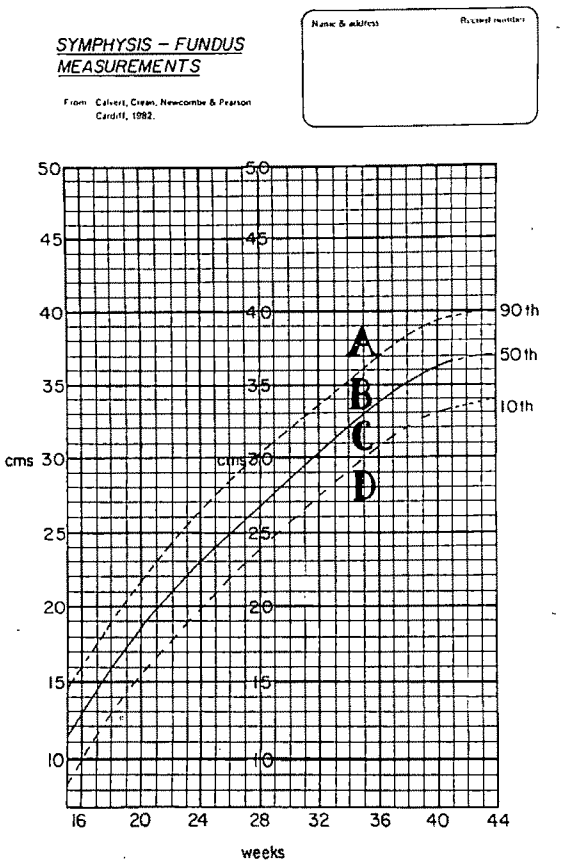


Fig. 1. Chart of symphysis-fundus height based on Cardiff data.[4]

tabulated, they were too few for statistical analysis; hence all analyses were restricted to groups A, B, and C. Birth weight and gestational age were analyzed by one-way analysis of variance.

Results

Primigravid patients (Table I). Because of the sample selection method, the distribution of symphysis-fundus heights was shifted to the right compared to published standards. There were 70 measurements in zone A, 195 in zone B, 122 in zone C, and 15 in zone D. Gestational age at delivery was slightly longer in group A than in groups B and C (Table II). Mean birth weight echeloned significantly from 3770 gm in group A, to 3410 gm in group B, and to 3170 gm in group C ($F = 51.7$, df = 2 and 384, $p < 0.001$).

The incidence of abnormal labor pattern (and/or second-stage delay) was higher in group A than in groups B and C ($\chi^2 = 29.1$, df = 2, $p < 0.001$). Similarly the incidence of operative delivery was greater in group A than in groups B and C ($\chi^2 = 31.8$, df = 2, $p < 0.001$). The corresponding rates of operative delivery for active-phase dystocia or second-stage delay in groups A, B, and C differ significantly ($\chi^2 = 44.0$, df = 2, $p < 0.001$).

Table I. Association of symphysis-fundus height with labor pattern and mode of delivery in 402 primigravid women in groups A, B, C, and D

	Group A (n = 70)		*Group B (n = 195)*		*Group C (n = 122)*		*Group D (n = 15)*	
	n	%	*n*	%	*n*	%	*n*	%
Labor pattern*								
Normal	23	33	124	64	87	71	12	80
Active-phase dystocia	27	39	52	27	28	23	1	7
Second-stage delay	28	40	42	22	16	13	2	13
Mode of delivery								
Spontaneous	20	29	117	60	85	70	8	53
Forceps	30	43	57	29	28	23	5	33
Cesarean section	20	29	21	11	9	7	2	13
Operative deliveries	N = 50		N = 78		N = 37		N = 7	
Indication for delivery								
Fetal distress	4	8	15	19	14	38	4	57
Active-phase dystocia/second-stage delay	42	84	58	74	22	58	3	43
Elective forceps	4	8	5	6	1	3	0	–

*Some patients had both active-phase dystocia and second-stage delay.

Table II. Mean gestational age and mean birth weight according to group in primigravid and multigravid women

	Group A	*Group B*	*Group C*	*Group D*
Primigravid women (n = 402)				
No. of measurements	70	195	122	15
Gestational age (wk)				
Mean	40.6	40.1	40.2	40.0
SD	1.3	1.5	1.2	1.6
Birthweight (gm)				
Mean	3770	3410	3170	2730
SD	430	390	400	440
Multigravid women (n = 518)				
No. of measurements	76	253	177	12
Gestational age (wk)				
Mean	39.9	40.1	40.0	40.4
SD	1.3	1.3	1.3	1.2
Birth weight (gm)				
Mean	3920	3520	3280	2753
SD	550	360	380	406

The proportion of operative deliveries for which the indication was fetal distress rather than poor progress increased progressively from group A mothers with larger infants (8%), through group B (19%), to group C mothers with smaller infants (38%) ($\chi^2 = 11.03$, df = 2, $p < 0.01$).

The influence of epidural analgesia was assessed (Table III). Epidural rates were similar in group A (30%), group B (27%), and group C (25%). In the 103 primigravid women with epidural analgesia, the expected increased incidence of second-stage delay and forceps delivery was observed. The increase in incidence was similar in extent regardless of group. However, in the 284 primigravid women not receiving epidural analgesia, there was a significantly higher incidence in group A of second-stage delay ($\chi^2 = 22.52$, df = 1, $p < 0.001$) and forceps delivery ($\chi^2 = 10.40$, df = 1, $p < 0.01$) than in group B. Group C was similar to group B.

The relationship between maternal height and operative delivery for active-phase dystocia or second-stage delay was examined (Table IV). The rate of operative delivery for dystocia in the short group was 42%, in the intermediate group 30%, and in the tall group 34% (short vs. intermediate: $\chi^2 = 4.00$, df = 1, $p < 0.05$). The high rate of operative delivery for active-phase dystocia or second-stage delay in group A primigravid women applied regardless of maternal height. Group B women, with average-sized infants, had a moderate risk if they were short or intermediate in stature and a low risk if tall. Group C women, with smaller babies, had a moderate risk if short and a low risk if intermediate or tall.

Multigravid patients (Table V). There were 76 mea-

Table III. Effect of continuous epidural analgesia on incidence of second-stage delay and forceps delivery according to symphysis-fundus group, in 387 primigravid women in groups A, B, and C

	Epidural analgesia (n = 103)						*No epidural analgesia (n = 284)*					
	Group A (n = 21)		*Group B (n = 52)*		*Group C (n = 30)*		*Group A (n = 49)*		*Group B (n = 143)*		*Group C (n = 92)*	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Second-stage delay	8	38	27	52	7	23	20	41	15	10	9	10
Forceps delivery	9	43	29	56	13	43	21	43	28	20	15	16

Table IV. Association of maternal height and symphysis-fundus height with rate of operative delivery for dystocia in 402 primigravid women

Symphysis-fundus group	*Maternal height*					
	<156 cm (n = 85)		*156-168 cm (n = 243)*		*>168 cm (n =74)*	
	*n**	%	*n**	%	*n**	%
A	12/18	67	16/29	55	18/23	78
B	16/39	41	43/122	35	4/34	12
C	8/26	30	12/79	15	3/17	18
D	0/2		3/13		—	

*Number of operative deliveries/total number of deliveries.

Table V. Association of symphysis-fundus height with labor pattern and mode of delivery in 518 multigravid women in groups A, B, C, and D

	Group A (n =76)		*Group B (n = 253)*		*Group C (n = 177)*		*Group D (n = 12)*
	n	%	*n*	%	*n*	%	*n*
Labor pattern*							
Normal	63	83	240	95	165	93	12
Abnormal	13	17	13	5	12	7	0
Active phase dystocia	11		9		7		
Second-stage delay	6		9		5		
Mode of delivery							
Spontaneous	63	83	232	92	168	95	11
Operative	13	17	21	8	9	5	
Forceps	9		13		6		1
Cesarean section	4		8		3		0

*Some patients had both active-phase dystocia and second-stage delay.

surements in zone A, 253 in zone B, 177 in zone C, and 12 in zone D. As with primigravid patients, gestational age at delivery in all groups was similar and mean birth weight decreased significantly from 3920 gm in group A to 3520 gm in group B and to 3280 gm in group C ($F = 65.5$; $df = 2$ and 503; $p < 0.001$) (Table II).

The well-known relative immunity of multigravid women to dystocia is illustrated (Table V). The pattern that emerged was similar to that found in primigravid women. Group A patients had a higher combined incidence of active-phase dystocia and second-stage delay (17%) than those in group B (5%) ($\chi^2 = 11.50$, $df = 1$, $p < 0.001$). The incidence in group C (7%) resembled that of group B. Group A patients also had a higher incidence of operative delivery (17%) than women in group B (8%) ($\chi^2 = 4.89$, $df = 1$, $p < 0.05$). The incidence in group C (5%) resembled that in group B.

Comment

Symphysis-fundus height measurement is a simple and readily communicable means of assessing fetal growth.[4] Our findings suggest that the symphysis-fundus height may also be of prognostic value with regard to pattern of labor and mode of delivery.

This study serves to quantify common sense. Group

A mothers (with larger infants) had a higher rate of abnormal labor and operative delivery than those mothers with smaller infants. This is consistent with Friedman's findings that increased fetal size is associated with a prolonged active phase and a progressive slowing in the maximum slopes of dilatation and descent.[6]

However, the extent of these problems among group A mothers was unexpected. The difference between group A mothers and mothers in groups B and C was gross for group A primigravid women and still considerable for group A multigravid women. It was even more pronounced when the confounding influence of epidural analgesia was removed. Thus group A primigravid women constitute a high-risk population, which requires delivery in a specialist obstetric unit.

Macrosomic infants have a high neonatal morbidity from delivery-associated complications. These include shoulder dystocia, meconium aspiration, birth asphyxia, and brachial plexus injury,[10] as well as the morbidity related to a higher incidence of midcavity forceps.[6] In our study 13 of 14 infants with birth weights >4500 gm were born to group A mothers. The alerting of the obstetrician to an increased risk of macrosomia before onset of labor could reduce high morbidity by earlier recourse to cesarean section.

Variable and late decelerations are of more serious import in growth-retarded fetuses,[11] who are at increased risk of intrapartum acidosis and asphyxia.[12] This study confirms that there is a progressive increase in the proportion of operative deliveries performed because of fetal distress, with decreasing size of the fetus. Antenatal fetal weight assessment by symphysis-fundus height recordings may alert the obstetrician to a higher probability of intrapartum fetal distress.

Obstructed labor remains commonplace in developing countries. Poor childhood nutrition may have led to poor maternal pelvic growth, but more recent socioeconomic improvement resulted in optimal fetal growth.[13] This considerable prevalence of cephalopelvic disproportion constitutes a major problem in association with a high domiciliary birth rate, in scattered communities, distant from specialist facilities.

There have been few antenatal screening procedures that predict for active-phase dystocia and second-stage delay. Both maternal height measurement[14, 15] and symphysis-fundus height measurement[4] are simple, reproducible, inexpensive screening tests associated with little loss of accuracy when performed by inexperienced attendents. However, although the mean height of women with major cephalopelvic disproportion was less than those without the disporportion, there was a wide range, and the individual patient's height could not be used as a predictive factor.[16]

In this study, women were grouped according to maternal height (as an indication of pelvic size) and symphysis-fundus height (as an indication of fetal size). It is important to note that the pregnancies at highest risk of dystocia were those involving larger infants (group A mothers), regardless of maternal height. Only when the infants were of average size (groups B and C) does tallness confer an advantage. Used alone, symphysis-fundus height measurement appeared to be a better discriminator than maternal height measurement with regard to the risk of dystocia. Used together, further information may be obtained on the relative risks of average-sized infants. The use of locally derived symphysis-fundus height nomograms could identify a high-risk primigravid population for early referral and result in an improved outlook for pregnant women with large babies.

REFERENCES

1. Westin B. Gravidogram and fetal growth. Acta Obstet Gynecol Scand 1977;56:273-82.
2. Belizan JM, Villar J, Nardin JC, Malamud J, de Vicuna LS. Diagnosis of intrauterine growth retardation by a simple clinical method: measurement of uterine height. AM J OBSTET GYNECOL 1978;131:643-6.
3. Quaranta P, Currell R, Redman CWG, Robinson JS. Prediction of small-for-dates infants by measurement of symphysial-fundal-height. Br J Obstet Gynaecol 1981;88: 115-9.
4. Calvert JP, Crean EE, Newcombe RG, Pearson JF. Antenatal screening by measurement of symphysis-fundus height. Br Med J 1982;285:846-9.
5. Studd JWW, Philpott RH. Partograms and action line of cervical dilatation. Proc Royal Soc Med 1972;65:700-1.
6. Friedman EA. Labor: clinical evaluation and management. 2nd ed. New York: Appleton-Century-Crofts, 1978.
7. Wood C, Ng KH, Hounslow D, Benning H. The influence of difference of birth times upon fetal condition in normal deliveries. J Obstet Gynaecol Br Common 1973;80: 289-94.
8. Pearson JF, Davies L. The effect of continuous lumbar epidural analgesia upon fetal acid-base status during the second stage of labour. J Obstet Gynaecol Br Common 1974;81:975-9.
9. Baird D. Dystocia due to faults in the pelvis. In: Clay A, ed. British Obstetric and Gynaecological Practice. vol. 1. London: Heinemann, 1963.
10. Modanlou HD, Dorchester WL, Thorosian A, Freeman RK. Macrosomia: maternal, fetal and neonatal implications. Obstet Gynecol 1980;55:420-4.
11. Lin C-C, Atef HM, Rosenow PJ, River P. Acid base characteristics of fetuses with intrauterine growth retardation during labor and delivery. AM J OBSTET GYNECOL 1980; 137:553-9.
12. Low JA, Pancham SR, Worthington D. Fetal heart deceleration pattern in relation to asphxyia and weight-gestational age percentile of the fetus. Obstet Gynecol 1976; 47:14-20.
13. Philpott RH. Recognition of cephalopelvic disproportion. Clin Obstet Gynaecol 1982;9:609-24.
14. Harrison KA. Cephalopelvic disproportion in the Third World. In: Crawford JW, ed. Risks of labour. Chichester, England: John Wiley and Sons, 1985.
15. Aitken IW, Karbo TK, Gba-Kamara AM. Planning a community-oriented midwifery service for Sierra Leone. World Health Forum 1985;6:110-4.
16. Stewart KS, Cowan DB, Philpott RH. Pelvic dimensions and the outcome of trial labour in Shona and Zulu primigravidas. S Afr Med J 1979;55:847-51.

Preterm labor: Stimulation of arachidonic acid metabolism in human amnion cells by bacterial products

Phillip R. Bennett, B.Sc., M.B., B.S., Mathew P. Rose, Ph.D., Leslie Myatt, Ph.D., and Murdoch G. Elder, M.D.

London, England

There is a strong association between preterm labor and infection. Some potentially pathogenic bacteria have phospholipase activity, and it has been suggested that release of phospholipase from these organisms may increase prostaglandin E_2 synthesis in amnion cells and hence initiate preterm labor. In this study we established monolayer amnion cell cultures from tissue collected at elective cesarean section at term before labor. Cells were prelabeled with tritiated arachidonic acid and then further incubated after addition of 2%, 5%, or 10% (vol/vol) filtered medium in which either group B β-hemolytic streptococcus, *Streptococcus viridans, Escherichia coli, Bacteroides fragilis,* or *Lactobacillus* had been growing. Tritiated arachidonic acid and its metabolites released by the amnion cells in these or control incubates were extracted from culture medium and separated by high-performance liquid chromatography. Addition of conditioned medium from each of the organisms with the exception of *Lactobacillus* caused an increase in overall arachidonic acid metabolism. There was an increase in the ratio of cyclooxygenase to lipoxgenase metabolism and in prostaglandin E_2 production in particular when compared to controls. The profile of arachidonic acid metabolism in amnion cells following addition of filtered bacterial medium resembled that obtained from amnion cells cultured following spontaneous labor. We suggest that abnormal bacterial colonization of the genital tract may lead to an increase in arachidonic acid metabolism in amnion cells with an increase in prostaglandin E_2 production and the consequent initiation of preterm labor. (AM J OBSTET GYNECOL 1987;156:649-55.)

Key words: Amnion, bacteria, preterm labor, prostaglandins

There is an association between preterm labor and infection. Several authors have reported a relationship between preterm rupture of membranes and maternal infection (reviewed in reference 1). Others have reported an association between preterm labor and infection with intact membranes and without signs or symptoms of infection.[2]

The commonest organism in the vagina is the *Lactobacillus,* but Goplerud et al.[3] have shown that pathogens such as *Escherichia coli, Streptococcus,* and *Bacteroides* can be isolated in small numbers from a high proportion of pregnant women. The general trend is for the numbers of these organisms to decrease as pregnancy progresses, with a resurgence during the puerperium.

Labor is associated with increased synthesis of prostaglandins by the fetal membranes, particularly the amnion.[4] These contain large amounts of arachidonic acid esterified in phospholipid pools which can be metabolized into various compounds including prostaglandins of the 2 series. Curbelo et al.[5] have measured the concentration of arachidonic acid in amnion and chorion at various stages of pregnancy and have found a concentration several times higher in amnion than in chorion, suggesting an important role for amnion in the initiation of labor. Several authors have suggested that term labor may be initiated by the activation of phospholipase enzymes that hydrolyze the esterified arachidonic acid, producing an increase in its free form and a consequent increase in prostaglandin synthesis.[6, 7] Bejar et al.[8] have shown that many pathogenic organisms may release a phospholipase enzyme, thereby activating arachidonic acid metabolism in the fetal membranes and initiating the biochemical processes of labor.

The principal cyclooxygenase metabolite of arachidonic acid in the amnion following labor is prostaglandin E_2. However, metabolism via lipoxygenase enzymes produces hydroxy–fatty acids.[9, 10] The role, if any, of these compounds in the physiology of human pregnancy has yet to be established.

In a pilot study Lamont et al.[11] have demonstrated that conditioned medium prepared from certain potentially pathogenic organisms increased the release of prostaglandin E_2, as measured by radioimmunoassay, from amnion cells in monolayer culture. We have

From the Institute of Obstetrics and Gynaecology, Hammersmith Hospital.

Received for publication January 16, 1986; revised September 9, 1986; accepted September 12, 1986.

Reprint requests: Dr. P. R. Bennett, Institute of Obstetrics and Gynaecology, Hammersmith Hospital, Du Cane Road, London, England W12.

Table I. Retention data for arachidonic acid metabolites on reverse-phase high-performance liquid chromatography

Compound	*Retention time (min)*	*Peak width (min)*
6-Keto-prostaglandin $F_{1\alpha}$	8.0	7.0-9.0
6-Keto-prostaglandin E_1	9.5	Not determined
Thromboxane B_2	11.0	10.5-12.0
Prostaglandin $F_{2\alpha}$	13.0	12.5-14.5
Prostaglandin E_2	16.5	15.5-18
Prostaglandin D_2	19.0	Not determined
13,14-Dihydro-15-keto-prostaglandin $F_{2\alpha}$	22.0	Not determined
13,14-Dihydro-15-keto-prostaglandin E_2	27.0	26.0-28.5
Prostaglandin A_2	29.0	Not determined
Prostaglandin B_2	31.5	Not determined
Leukotriene B_4	34.0	34.0-35.0
5-Hydroxyeicosatetraenoic acid (two peaks)	35.5	34.5-36.5
	43.0	42.0-44.0
8-Hydroxyeicosatetraenoic acid	42.5	41.5-43.0
9-Hydroxyeicosatetraenoic acid	44.0	43.0-45.0
11-Hydroxyeicosatetraenoic acid	42.5	43.0-44.0
12-Hydroxyeicosatetraenoic acid (two peaks)	35.5	35.0-36.0
	42.5	41.5-43.0
15-Hydroxyeicosatetraenoic acid	42.0	41.5-43.0
Arachidonic acid	58.0	57.5-58.5

Authentic tritiated or carbon-labeled standards were fractionated on a Waters fatty acid analysis column with use of a stepwise gradient of 30%, 50%, and 100% acetonitrile in 40 mmol/L of triethylamine formate (pH 3.15) at a flow rate of 1 ml/min for 25, 25, and 8 minutes, respectively. Retention times at maximum peak height and the peak widths are given.

sought to investigate the effect of these organisms on the full range of arachidonic acid metabolism by the amnion and to demonstrate dose-related changes in both cyclooxygenase and lipoxygenase products.

Material and methods

Bacteria were grown in suitable culture medium for 24 hours and then removed by filtration. Amnion cells in monolayer culture were incubated with tritiated arachidonic acid to allow incorporation into esterified pools, and the bacterial filtrate was added to them in various concentrations. Metabolites of tritiated arachidonic acid released by the amnion cells were separated by high-performance liquid chromatography and comparison made with metabolism in control cultures.

Bacterial preparations. Stock isolates of *E. coli*, Lancefield group B hemolytic streptococcus, *S. viridans*, *B. fragilis* and *Lactobacillus* were inoculated into 50 ml of suitable culture medium (Oxoid No. 2 broth or Difco Thiol liquid broth 1723/37). These isolates were incubated for 24 hours at 37° C, resulting in 10^8 organisms per milliliter of medium, and the organisms were separated by centrifugation and filtration. The conditioned medium was then stored in aliquots at −20° C until required.

Amnion cell cultures. Complete placentas and membranes were obtained from women having elective cesarean sections at term, who had not been in labor and who had had uncomplicated pregnancies, or after spontaneous labor at either term or at 30 weeks. The membranes were cut from the placental edge and the amnion was carefully peeled from the chorion by hand. The amnion, bathed in phosphate-buffered saline solution (PBS), was cut into strips 1 by 5 cm and washed twice in PBS. The strips were incubated at 20° C for 15 minutes in 0.5 mmol/L ethylenediaminetetraacetate in PBS, washed twice in PBS, and then incubated in a solution of Dispase grade II (2.5 gm/L in PBS; Boehringer) at 37° C for 45 minutes. The strips were carefully removed into 100 ml of tissue culture medium 199 (Gibco) with 10% horse serum, and the epithelial cells were liberated by vigorously shaking the container for 3 minutes. The amnion strips were then removed, an aliquot of 0.3 ml of the cell suspension was added to 0.1 ml of trypan blue, and the number of viable cells excluding the dye were counted in a hemocytometer cell. The cell concentration was then adjusted so that 10^6 cells were added to each well of a multiwell tissue plate (Flow Laboratories 76-053-05) in 2 ml of medium. This number of cells leads to a confluent homogenous cellular monolayer within 24 hours. The cells were then incubated at 37° C in an atmosphere of 5% carbon dioxide in air.

After 24 hours the culture medium was removed together with any nonadherent cells. Tritiated arachidonic acid (155 Ci/mmol; Amersham International) was added to fresh culture medium to an activity of 0.2 μCi/ml and 2 ml of this added to each culture well. After incubation for a further 24 hours (during which some metabolism of tritiated arachidonic acid takes

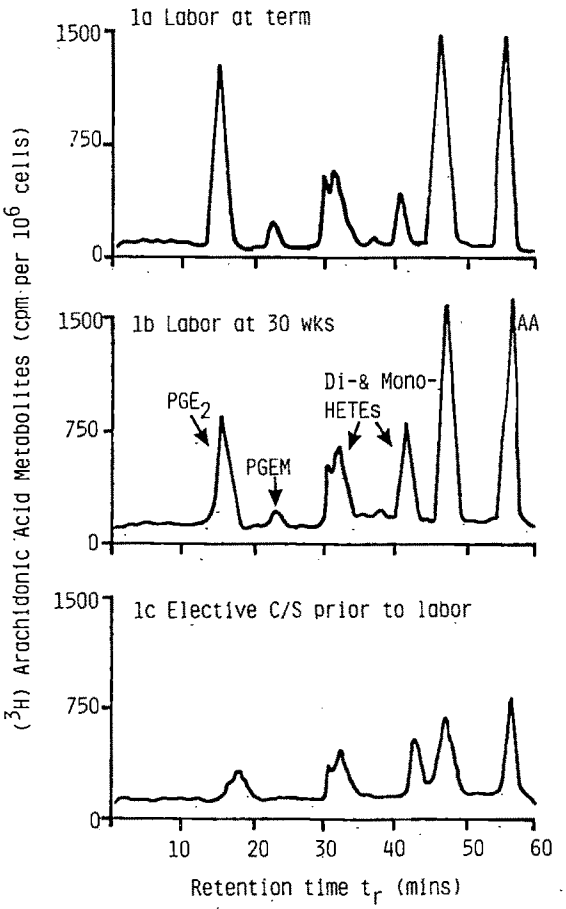


Fig. 1. Representative high-performance liquid chromatography profiles of tritiated arachidonic acid metabolites released by prelabeled amnion cells in tissue culture. Cells were obtained after spontaneous labor at term *(1a)*, spontaneous labor at 30 weeks *(1b)*, and elective cesarean section at term before labor *(1c)*. HETE = Hydroxyeicosatetraenoic acid.

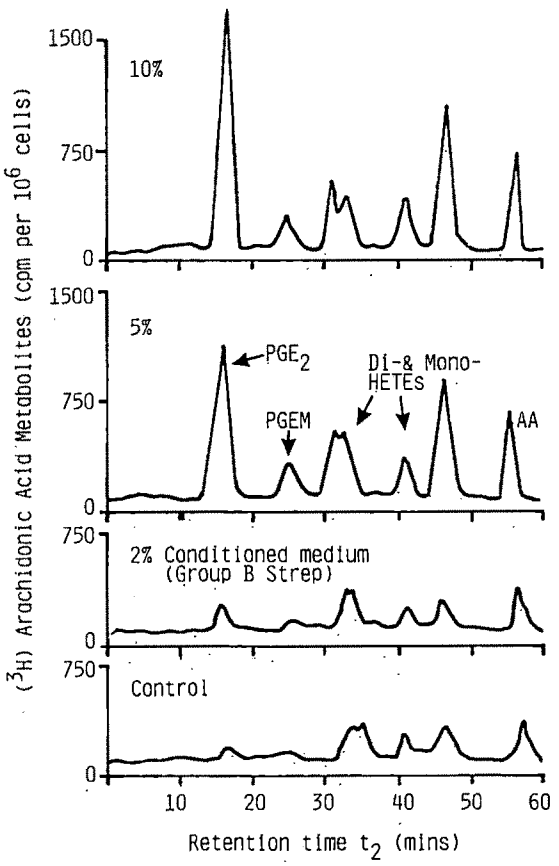


Fig. 2. Representative high-performance liquid chromatography profiles of tritiated arachidonic acid metabolites released by prelabeled amnion cells in tissue culture. Cells were obtained after elective cesarean section at term before labor. After labeling, cells were incubated with 10% *(first panel)*, 5% *(second panel)*, 2% *(third panel)*, and 0% *(fourth panel)* group B streptococcus conditioned medium.

place, but most is retained within the cells, esterified within phospholipid pools[12]), this medium was removed and added to scintillation fluid (Unisolve 1, Koch-Light Ltd.), and radioactivity was counted to allow calculation of arachidonic acid uptake by the cells. The cells were washed twice with PBS, and 2 ml of fresh culture medium with 10% horse serum without tritiated arachidonic acid but containing 2%, 5%, and 10% (vol/vol) bacterial filtrate was added to duplicate wells. Similar preparations containing 10% unused sterile bacterial culture medium were used as controls. Any change in pH of the culture fluid was adjusted for by titration with 7.5% sodium bicarbonate before use. After a further 24 hours incubation during which tritiated arachidonic acid may have been released from esterified pools and metabolized, the medium was removed and that from duplicate wells was pooled and stored at −20° C until analysis. Each well was stained with trypan blue to confirm viability of the cells.

Analysis by high-performance liquid chromatography. Each 4 ml sample of medium was acidified to a pH of 3.15 with formic acid and passed through a prepared C_{18} SepPak (Waters Associates), and the tritiated arachidonic acid metabolites were eluted into 5 ml of methyl formate. The solvent was evaporated with oxygen-free nitogen and the residue redissolved in 1 ml of 30% (vol/vol) acetonitrile in 40 mmol/L of triethylamine formate (pH 3.15). The fatty acids were then separated according to polarity with use of a high-pressure liquid chromatography system based on a reverse-phase fatty acid analysis column (Waters Associates). The initial solvent mixture was 30% acetonitrile in 40 mmol/L triethylamine formate for 25 minutes, increasing to 50% and 100% acetonitrile after 25 and 52 minutes, respectively. The solvent flow was 1 ml/min, and 0.5 minute fractions were collected for a total of 60 minutes. Each fraction was dissolved in 4 ml of scintillation fluid and the radioactivity counted in an Intertechnique SL3000 liquid scintillation counter.

We have previously reported the elution times for

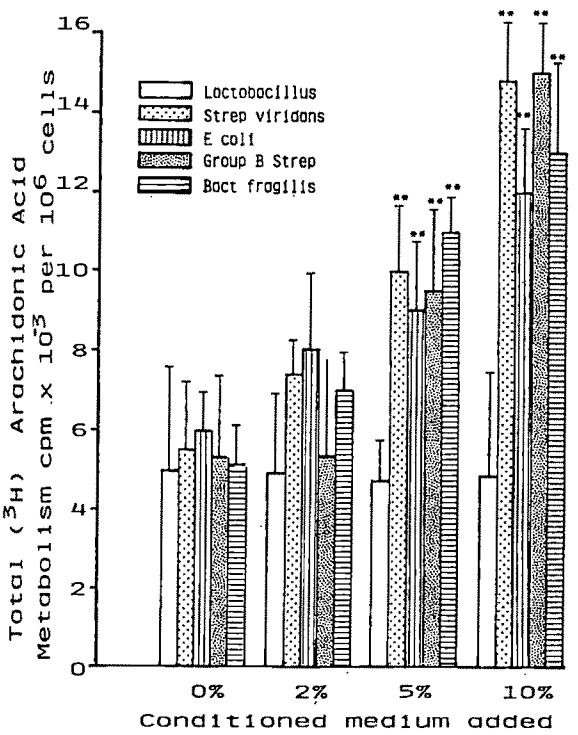


Fig. 3. Total tritiated arachidonic acid metabolism from prelabeled amnion cells cultured after elective cesarean section at term before labor when incubated with various concentrations of conditioned medium prepared from each organism. Mean values ±SD are shown for three separate experiments. Significant differences from control: * = $p < 0.05$, ** = $p < 0.01$ (analysis of variance).

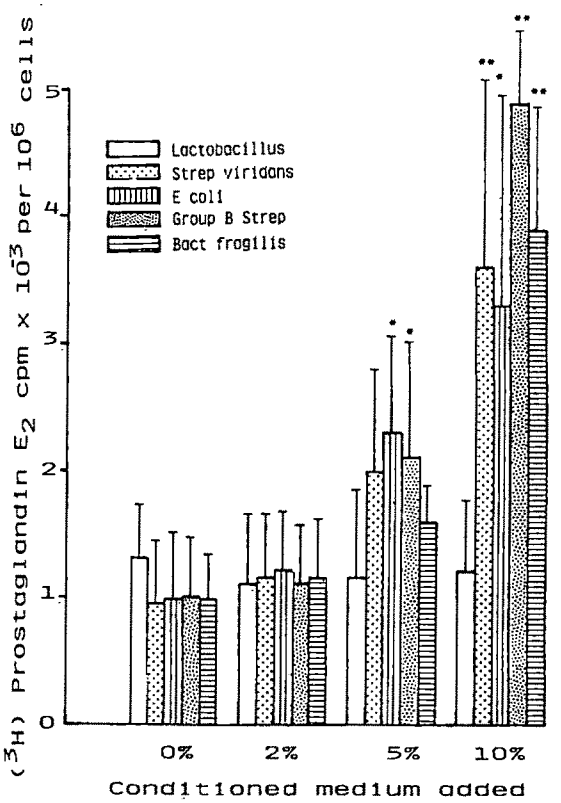


Fig. 4. Prostaglandin E_2 metabolism from tritiated arachidonic acid in prelabeled amnion cells cultured after elective cesarean section at term before labor when incubated with various concentrations of conditioned medium prepared from each organism. Mean values ±SD are shown for three separate experiments. Significant differences from control: * = $p < 0.05$, ** = $p < 0.01$ (analysis of variance).

various arachidonic acid metabolites in this system[13] (Table I). The radioactivity present in each metabolite peak in the high-performance liquid chromatography profiles was calculated by summation. Each experiment was repeated three times, and the significance of metabolite production on addition of conditioned medium was compared with controls for each organism with use of analysis of variance.

Results

The uptake of tritiated arachidonic acid by amnion cells during 24 hours of culture was 77% ± 3.5 (mean ± SD) and was not significantly different between wells from a single tissue sample or between different samples of amnion whether cells were collected at elective cesarean section or after labor. At the end of each experiment more than 97% of the cells remained viable. Metabolism of tritiated arachidonic acid was not significantly different in those cells cultured with 10% unused sterile bacterial culture medium when compared with those cultured in tissue culture medium 199 with 10% horse serum without bacterial culture medium.

We have demonstrated release of compounds that cochromatograph with prostaglandin E_2, its metabolite 13,14-dihydro-15-keto-prostaglandin E_2, leukotriene B_4, 5-hydroxyeicosatetraenoic acid, and 12-hydroxyeicosatetraenoic acid. The compound that elutes at 46 minutes has not been identified, although on the basis of its relative lack of polarity and its acid stability, it may be a fatty acid epoxide produced by cytochrome P_{450} enzymes as has been described by Chacos et al.[14]

The high-performance liquid chromatography profile of metabolites arising from the endogenously incorporated tritiated arachidonic acid from amnion cells cultured after spontaneous labor at term (Fig. 1, *a*) was compared with the profiles from amnion cells obtained following spontaneous labor at 30 weeks (Fig. 1, *b*) or following cesarean section at term before labor (Fig. 1, *c*). There was an apparent increase in total metabolism of tritiated arachidonic acid after labor, with increased release of prostaglandin E_2 (retention time, or t_r = 16 minutes), dihydroxy–fatty acids (dihydroxyeicosatetraenoic acid) co-chromatographing with leukotriene B_4 (t_r = 34 minutes) and monohydroxy–fatty acids (monohydroxyeicosatetraenoic acids,

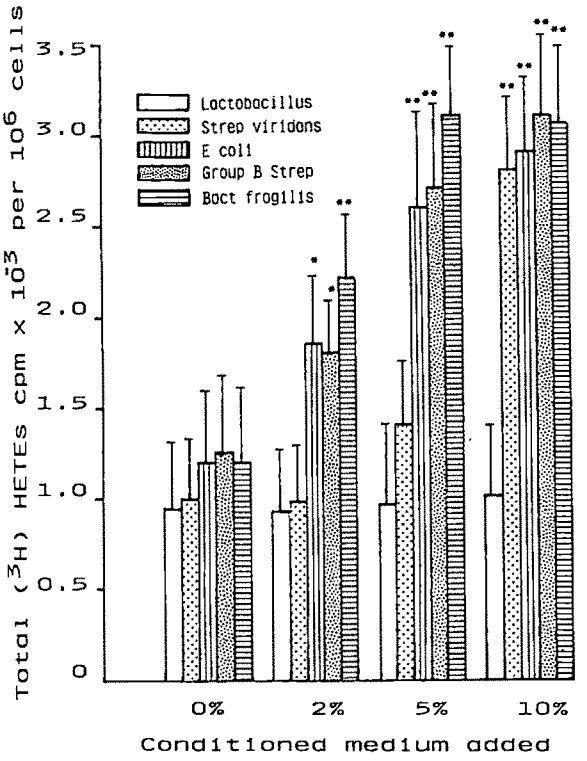


Fig. 5. Dihydroxyeicosatetraenoic and monohydroxyeicosatetraenoic acid metabolism from tritiated arachidonic acid in prelabeled amnion cells cultured after elective cesarean section at term before labor when incubated with various concentrations of conditioned medium prepared from each organism. Mean values ±SD are shown for three separate experiments. Significant differences from control: * = $p < 0.05$, ** = $p < 0.01$ (analysis of variance).

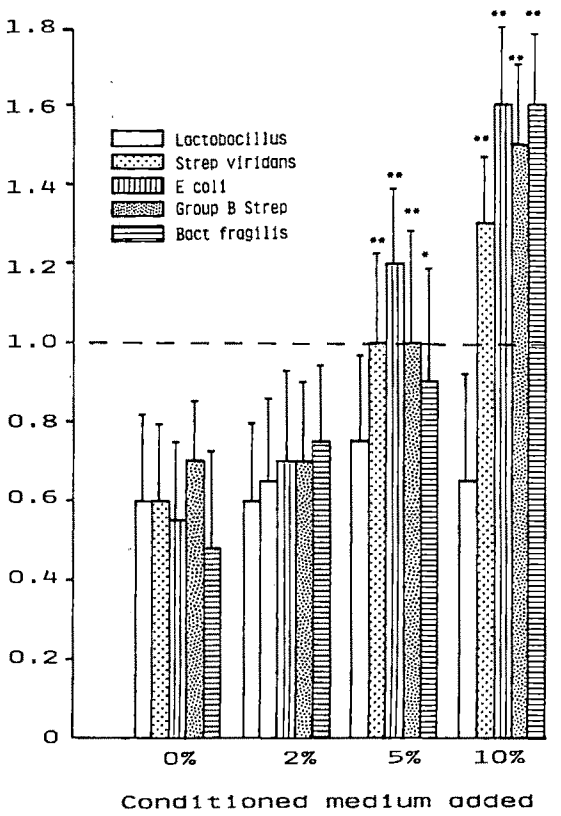


Fig. 6. Ratio of cyclooxygenase (prostaglandin E_2) to lipoxygenase (dihydroxyeicosatetraenoic acid and monohydroxyeicosatetraenoic acid) metabolism from tritiated arachidonic acid in prelabeled amnion cells cultured after elective ceserean section at term before labor when incubated with various concentrations of conditioned medium prepared from each organism. Mean values ±SD are shown for three separate experiments. Significant differences from control: * = $p < 0.05$, ** = $p < 0.01$ (analysis of variance).

t_r = 35 and 43 minutes). After either term or preterm labor there was a change in the proportion of tritiated arachidonic acid metabolism from lipoxygenase to cyclooxygenase products and therefore a relative increase in prostaglandin E_2 synthesis (Fig. 1, *b* and *c*). These changes appear to be related to the parturition process rather than the length of the pregnancy, since amnion obtained after labor at 30 weeks also had a considerable ability to synthesize prostaglandin E_2.

High-performance liquid chromatography profiles from a single experiment in which amnion cells obtained after elective cesarean section and prelabeled with tritiated arachidonic acid were incubated with increasing concentrations of group B streptococcus–conditioned medium are shown in Fig. 2. Summation of radioactivity within the peaks on the high-performance liquid chromatography profiles revealed an increase in total tritiated arachidonic acid metabolism with an apparent dose-related increase in prostaglandin E_2 production on the addition of increasing amounts of conditioned medium. Addition of 5% and 10% (vol/vol) conditioned medium prepared from all of the organisms tested except that prepared from *Lactobacillus* caused a significant increase in total tritiated arachidonic acid metabolism from endogenous sources (Fig. 3). The four active organisms had similar concentration response curves. There was an increase in metabolism via both cyclooxygenase (Fig. 4) and lipoxygenase (Fig. 5) pathways to prostaglandin E_2 and hydroxyeicosatetraenoic acids, respectively. However, the increase in cyclooxygenase metabolism was greater than the increase in lipoxygenase metabolism and the cyclooxygenase/lipoxygenase product ratio increased with addition of conditioned medium from the four active organisms (Fig. 6). Production of the prostaglandin E_2 metabolite, 13,14-dihydro-15-keto-prostaglandin E_2 (t_r = 27 minutes) was not increased despite an increase in release of prostaglandin E_2 itself. Increasing the concentration of conditioned medium to 20% caused no further increase in tritiated arachidonic acid metabolism but reduced the viability of the cells at the end of the experiment.

Comment

In this in vitro study we have used amnion cells prelabeled with tritiated arachidonic acid to replicate the in vivo source of arachidonic acid in esterified pools. Myatt et al.[9] have demonstrated that incubation with exogenous arachidonic acid causes release of prostaglandin E_2 whether the cells have been obtained before or after labor.

We have attempted to examine the full range of arachidonic acid metabolism in human amnion cells and have demonstrated metabolism by both cyclooxygenase and lipoxygenase enzymes. Our finding that the only prostaglandins produced by amnion cells are prostaglandin E_2 and its metabolite 13,14-dihydro-15-keto-prostaglandin E_2 agrees with the findings of Kinoshita and Green,[10] who were unable to demonstrate production of significant quantities of prostaglandin $F_{2\alpha}$, prostaglandin I_2, or 6-keto prostaglandin $F_{1\alpha}$ from homogenized amnion. We cannot exclude metabolism of prostaglandin E_2 to 13,14-dihydro-15-keto-prostaglandin E_2 by enzymes introduced by the horse serum. Production of 5-hydroxyeicosatetraenoic acid and 12-hydroxyeicosatetraenoic acid has been demonstrated by Kinoshita and Green[10] and Myatt et al.,[9] both of whom have confirmed their chromatographic evidence by gas chromatography and mass spectrometry.

There is an increase in arachidonic acid metabolism associated with both preterm and term labor with a change in the ratio of cyclooxygenase to lipoxygenase metabolism and a large increase in the production and release of prostaglandin E_2.[13] Similar findings have previously been reported in rabbits.[13] It has been suggested that, at term, this may be due to an increase in phospholipase enzyme activity.[6] Prostaglandin E_2 probably has a major role in the initiation of labor.

We have shown that potentially pathogenic organisms appear to cause an increase in arachidonic acid metabolism in amnion cells with an increase in prostaglandin E_2 release and a change in the ratio of cyclooxygenase to lipoxygenase metabolism, whereas *Lactobacillus*, which forms the normal flora of the vagina, does not. This effect is dose related, and the dose response curves for each organism are similar, which suggests a common mechanism. It is possible that these organisms exert this effect by release of phospholipase, since there is an increase in both cyclooxygenase and lipoxygenase metabolism. It is unlikely that these organisms release the cyclooxygenase enzyme, since Gulbis et al.[12] have demonstrated that they do not synthesize prostaglandin E_2 themselves. We have shown (unpublished work) that these organisms are unable to metabolize either cyclooxygenase or lipoxygenase products from exogenous tritiated arachidonic acid. The shift from predominately lipoxygenase to cyclooxygenase metabolism may be a consequence of the biochemistry of the amnion cell, since similar changes are seen in association with normal labor. However, it is possible that bacteria release other substances that have been shown to stimulate arachidonic acid metabolism in amnion.[16] The lack of increase in prostaglandin E_2 metabolite release despite increase in prostaglandin E_2 release is in keeping with the evidence that amnion is a minor site of further metabolism of prostaglandins in comparison with chorion and decidua, which contain larger amounts of prostaglandin dehydrogenase.[4]

This study strongly suggests a role for certain bacteria in the etiology of preterm labor in the presence of intact membranes. It explains the onset of labor in the presence of overt chorioamnionitis and may also explain the rapid onset of labor before or at term following premature rupture of fetal membranes with consequent bacterial colonization. Further work is needed to identify the bacterial agents responsible, and the role of the lipoxygenase products needs to be delineated. It should be possible, with use of a system similar to that described here, to demonstrate the effect of bacteria on the catabolism of prostaglandins in chorion and decidua. Such work would increase our understanding of the etiology of both preterm and term labor.

We are grateful to Glaxo Ltd. for providing a perinatal research fellowship for Dr. P. R. Bennett and to David Adams, Department of Bacteriology, Hammersmith Hospital, for providing bacterial isolates.

REFERENCES

1. Varnier MW, Galansk RP. Preterm premature rupture of membranes. In: Elder MG, ed. Preterm labour. London: Butterworths, 1981.
2. Bobitt R, Ledger W. Unrecognised amnionitis and prematurity. J Reprod Med 1977;19:9.
3. Goplerud CP, Ohmm J, Galansk RP. Aerobic and anaerobic flora of the cervix in pregnancy and the puerperium. AM J OBSTET GYNECOL 1976;126:858.
4. Skinner KA, Challis JRG. Changes in the synthesis and metabolism of prostaglandins by human fetal membranes and decidua at labor. AM J OBSTET GYNECOL 1985; 151:519.
5. Curbelo V, Bejar R, Benirschke K. Prostaglandin precursors in human placental membranes. Obstet Gynecol 1981;57:473.
6. Okazake T, Okita J, Macdonald PC. Initiation of human parturition. AM J OBSTET GYNECOL 1978;130:432.
7. Greivy S, Liggins G. Phospholipase activity in human and ovine uterine tissue. Prostaglandins 1976;12:229.
8. Bejar R, Curbello V, Davis C, Gluck L. Premature labour and bacterial sources of phospholipase. Obstet Gynecol 1981;57:473.
9. Myatt L, Rose MP, Elder MG. Lipoxygenase products of arachidonic acid in human fetal membranes [Abstract]. Phoenix, Arizona: Society of Gynecological Investigation, thirty-second annual meeting, 1985:160.
10. Kinoshita K, Green K. Bioconversion of arachidonic acid and related compounds in human amnion. Biochem Med 1980;23:185.
11. Lamont RF, Rose MP, Elder MG. Effect of bacterial prod-

ucts on prostaglandin E production by amnion cells. Lancet 1985;2:1331.
12. Gulbis E, Galand N, Dumont JE. Prostaglandin formation in bacteria: a reappraisal. Prostaglandins 1981;21:439.
13. Rose MP, Elder MG, Myatt L. Arachidonic acid metabolism in fetal membranes and placenta: trophoblast research (in press).
14. Chacos N, Falck JR, Wixtrom C, Capdevila J. Novel epoxides formed during the liver cytochrome P-450 oxidation of arachidonic acid. Biochem Biophys Res Comm 1982;104:916.
15. Eliott WJ, Mclaughlin LL, Bloch MH, Needleman P. Arachidonic acid metabolism by rabbit fetal membranes of various gestational ages. Prostaglandins 1984;27:27.
16. Challis J. The role of prostaglandins in labour. International Congress and Symposium Series of the Royal Society of Medicine. London: Royal Society of Medicine, 1985;92:5.

Umbilical artery Doppler blood flow studies in the preterm, small for gestational age fetus

Lesley M. McCowan, M.B., Lawrence A. Erskine, M.B., and Knox Ritchie, M.D.
Toronto, Ontario, Canada

Umbilical artery Doppler studies were performed on the day of delivery in 15 singleton preterm pregnancies. In each case the fetus was significantly growth retarded on ultrasound examination and the attending obstetrician had made a decision to deliver the patient of the infant. Eight women had severe preeclampsia, three had mild chronic hypertension, and in four there was no recognized maternal disorder. Doppler studies were analyzed by use of the pulsatility index and the A/B ratio. In 14 of the 15 cases both were markedly abnormal. Three early neonatal deaths occurred. A positive correlation was found between the degree of abnormality of the pulsatility index and A/B ratio and a poor neonatal outcome ($p < 0.001$ and $p < 0.02$). Umbilical artery Doppler studies were not helpful in differentiating between intrinsic and extrinsic causes of growth retardation but seemed to identify the pregnancy at increased risk of a poor neonatal outcome. (Am J Obstet Gynecol 1987;156:655-9.)

Key words: Doppler, umbilical artery, flow velocity waveforms, intrauterine growth retardation

Severe intrauterine growth retardation (IUGR) recognized in the second or early third trimester is uncommon and can be of extrinsic or intrinsic origin. In the former case it is most often associated with severe preeclampsia.[1] In the absence of maternal disease, an intrinsic etiology such as fetal infection or chromosome abnormality may be suspected. In as many as one third of pregnancies complicated by IUGR no predisposing cause can be identified.[2] Although the diagnosis of IUGR by ultrasound has become increasingly sensitive it is not generally useful in discerning the cause of growth failure.[3]

In early onset IUGR it can be difficult to assess fetal well-being by means of the nonstress test because the fetus is immature and the mother may be receiving medications that reduce fetal heart rate variability.[4] Furthermore, the growth-retarded fetus may have reduced heart rate variability even in the absence of acidosis.[5] It can therefore be difficult to time intervention.

The application of Doppler ultrasound to the study of fetal umbilical arterial blood flow velocity is a relatively new technique first described by Fitzgerald and Drumm[6] in 1977. Previous studies demonstrated that the end-diastolic velocity in other arterial beds is inversely related to peripheral resistance.[7] In normal pregnancy the umbilical circulation has low resistance and there is forward flow velocity throughout the cardiac cycle. As gestation advances end-diastolic velocity increases progressively, indicating a gradual fall in resistance in the umbilical-placental circulation.[8]

Previous studies have shown that substantially reduced or absent end-diastolic flow velocity in the severely growth-retarded fetus is associated with a risk of intrauterine death[8] or 5-minute Apgar score of <6.[9] The degree of flow velocity waveform abnormality, however, was not found to be related to outcome. In neither of these studies were observations made within 24 hours of delivery.

From the University of Toronto, Perinatal Complex.
Supported by Grant No. 85-50 from the Hospital for Sick Children Foundation, Toronto.
Received for publication June 13, 1986; revised September 8, 1986; accepted October 29, 1986.
Reprint requests: J. W. K. Ritchie, Department of Obstetric Perinatology, Mt. Sinai Hospital, 600 University Ave., Toronto, Ontario, Canada M5G 1X5.

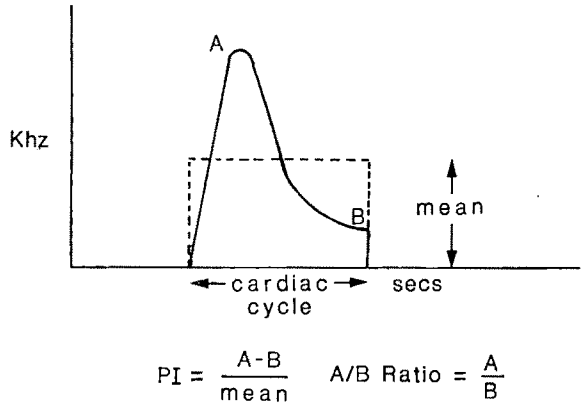


Fig. 1. Schematic representation of a flow velocity waveform showing methods of calculation of pulsatility index and A/B ratio.

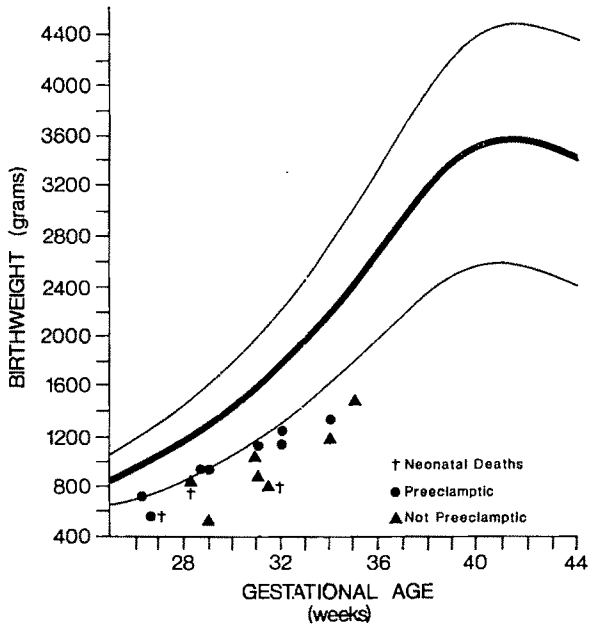


Fig. 2. Birth weight distribution of the neonates. Normal range (mean ± 2 SD) is shown.

The principal aims of this study were to determine whether the umbilical arterial flow velocity waveform in fetuses with early onset of IUGR could be related to fetal condition at birth and to determine whether this waveform was useful in differentiating between intrinsic and extrinsic causes of growth retardation.

Patients and methods

Umbilical artery Doppler flow velocity studies were performed in 15 preterm patients with severe IUGR suggested by ultrasound examination and confirmed at birth. In all cases examinations were performed on the day of delivery. None of the Doppler study results were made known to the attending physician and did not influence patient management.

Patients. The mean age of patients studied was 28 years (range 21 to 39). Nine patients were nulliparous. Eight patients were diagnosed as having preeclampsia and three as having mild chronic hypertension. In four instances there was no identifiable maternal abnormality.

Methods. Examinations were performed with a portable continuous wave instrument (Bach-Simpson, London, Ontario, Canada) with a 4 MHz probe (power output at the transducer surface 80 mW/cm^2). Most examinations were performed at the bedside with the patient in a semirecumbent position, and when the characteristic auditory signal of the umbilical artery was obtained it was recorded by a tape recorder (Sony Professional Walkman). These data were played back through a spectral analyzer (Advanced Technology Laboratories, Bothell, Washington) and a hard-copy printout was made to include at least 10 waveforms for subsequent secondary analysis.

Evidence from peripheral vascular studies suggests that end-diastolic velocity reflects peripheral resistance and several different indices have been used to express changes in resistance. Methods used to analyze umbilical artery time velocity waveforms have been described in detail.[8] The most useful appears to be the pulsatility index because it can quantify results adequately when diastolic flow velocity is absent or reversed. The A/B ratio is also useful and simple to calculate but has the disadvantage of becoming infinity when flow velocities are absent or negative when they are reversed. Both ratios were computed in the present study; the method of calculating them is shown in Fig. 1. Ten consecutive waveforms were analyzed and the mean result was compared with our previously established normal range.[8] In an attempt to identify the fetus that might be at increased risk of a poor outcome, an arbitrary pulsatility index value of 2 was chosen. All values above this level had absent or reversed end-diastolic flow velocity. Outcomes were compared in infants with pulsatility index values above and below 2.

To perform a similar analysis for the A/B ratio, a value of >100 was chosen because this included all cases with absent end-diastolic flow velocity. One case with reversed end-diastolic flow velocity was also included in this group.

All of the patients had reliable menstrual dates and had an ultrasound examination in the first or second trimester.

Usher and McLean[9] charts were used to classify the birth weight of the infants studied. Growth retardation was diagnosed when birth weight was 2 SD below the mean.

The results were subjected to statistical analysis that

Table I. Maternal and neonatal characteristics of the patients studied

Case No.	*Concurrent maternal disorder*	*Maternal age (yr)*	*Gestation (wk)*	*Birth weight (gm)*	*End-diastolic velocity*	*Pulsatility index*	*Umbilical artery pH*	*Outcome*
1	Severe preeclampsia, ruptured liver	30	34	1330	Normal	1.10	7.01	Well
2	Severe preeclampsia	32	26	570	Absent	2.40	7.17	Early neonatal death
3	Severe preeclampsia	29	28	860	Reduced	1.47	7.34	Well
4	Severe preeclampsia	26	26	770	Absent	1.84	7.33	Well
5	Severe preeclampsia	32	29	910	Reduced	1.88	7.35	Alive, dilated ventricles
6	Severe preeclampsia	30	32	1240	Absent	2.43	7.22	Well
7	Severe preeclampsia	26	32	1120	Reduced	1.79	7.21	Well
8	Severe preeclampsia	28	31	1140	Reduced	1.40	7.32	Well
9	None	36	31	820	Reduced	1.46	7.29	Well
10	Mild hypertension	38	31	790	Absent	2.53	7.15	Early neonatal death
11	Mild hypertension	21	34	1190	Reduced	1.80	7.29	Well
12	None	25	29	580	Absent	2.78	7.23	Possible congential viral infection
13	None	39	35	1510	Absent	1.93	7.28	Well
14	Mild hypertension	30	28	850	Reversed	8.10	7.22	Early neonatal death
15	None	32	31	1040	Absent	3.16	7.30	Trisomy 21

included the calculation of correlation between variables and the Mann-Whitney one-tailed nonparametric test.

Results

Thirteen patients were delivered electively by cesarean section. On two occasions this was done for maternal indications (worsening preeclampsia) and the remaining 11 for presumed serious fetal compromise.

The mean birth weight at delivery was 981 gm (range 570 to 1510 gm) and the mean gestational age 30.7 weeks (range 26 to 35 weeks). There were three early neonatal deaths. In all cases birth weight was at least 2 SD below the mean (Fig. 2). Further details of the neonates including Apgar scores, umbilical artery pH, and the need for intubation at birth and the antepartum pulsatility index values are shown in Table I. Outcome refers to condition at discharge from hospital. A good outcome was defined as an infant that was well and appeared neurologically normal at the time of discharge from the hospital. A poor outcome was defined as neonatal death, chromosomal abnormality, or evidence of a neurological problem at the time of discharge from hospital. By these criteria nine infants had good outcomes and six had poor outcomes. No newborn infant had a hematocrit >60%.

Successful Doppler studies were possible in all of the patients. In 14 of the 15 cases studied, the pulsatility index and A/B ratios were substantially outside the normal range. The values obtained for the last pulsatility index and A/B ratios are shown in Figs. 3 and 4. The A/B ratio in the single patient with reversed end-diastolic flow velocity is reported as >8.

Neonatal outcome was examined in all cases. There was no correlation between umbilical artery pH, Apgar scores, or the need for intubation at birth and the degree of abnormality of either the pulsatility index or A/B ratio. The six patients with an abnormal pulsatility index value had either absent (five cases) or reversed (one case) end-diastolic flow velocity. This group included the three infants that died in the early neonatal period and a case of trisomy 21. In one case congenital viral infection was suspected because of hepatosplenomegaly, hyperbilirubinemia, intracerebral calcification, and abnormal muscle tone. There was one apparently healthy infant. Five of these six cases therefore had a poor outcome. The infants with poor outcomes had significantly higher pulsatility index values than those with a good outcome ($p < 0.001$, one-tailed Mann-Whitney test).

There were eight infants with an A/B value of >100. This group included the three that died, the infant with trisomy 21, and one with suspected congenital viral infection. Three infants were apparently normal. Thus five of the eight had a poor outcome. The infants with poor outcomes had significantly higher A/B values than those with good outcomes ($p < 0.02$, one-tailed Mann-Whitney test).

Only three infants in the series were acidotic at birth, with an umbilical artery pH <7.20. Two of the three subsequently died. The surviving infant (Case No. 1) had a normal umbilical artery end-diastolic flow velocity and the low cord pH probably resulted from acute maternal blood loss resulting from a ruptured liver in a woman with severe preeclampsia.

The first of the three infants that died (Case No. 2) was born at 26.7 gestational weeks, developed severe respiratory distress syndrome, and died of respiratory complications and renal failure. The second (Case No. 10) was born at 31.4 gestational weeks, had a pul-

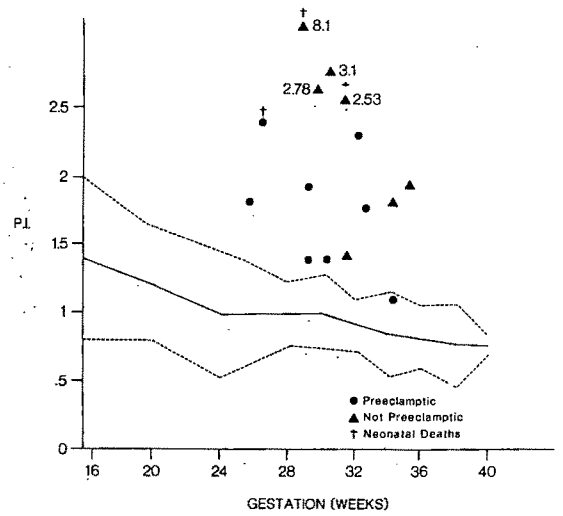


Fig. 3. Last pulsatility index measurements. Normal range (mean ± 2 SD) is shown.

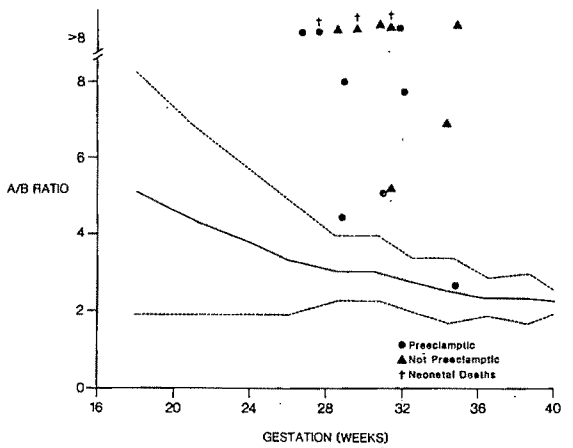


Fig. 4. Last A/B ratio measurements. Normal range (mean ± 2 SD) is shown.

monary hemorrhage at 3 days, and an intracerebral hemorrhage was found at autopsy. The third (Case No. 14) was born at 28.3 gestational weeks, developed respiratory distress syndrome and had a pulmonary hemorrhage, and was found to have intracerebral hemorrhage at autopsy. Each of the three babies that died was thrombocytopenic at birth and this is the subject of a further study.

All of the patients had a nonstress test performed. It was reactive in four and there was a good outcome in each case. Eleven patients had a nonreactive nonstress test with poor fetal heart rate variability. Five of the mothers were receiving magnesium sulfate infusions and six were receiving no medication. Three of the 11 had low umbilical pH (<7.20) at birth, including the two infants with very abnormal pulsatility index values who subsequently died and one whose mother was found to have a ruptured liver. Of the remaining

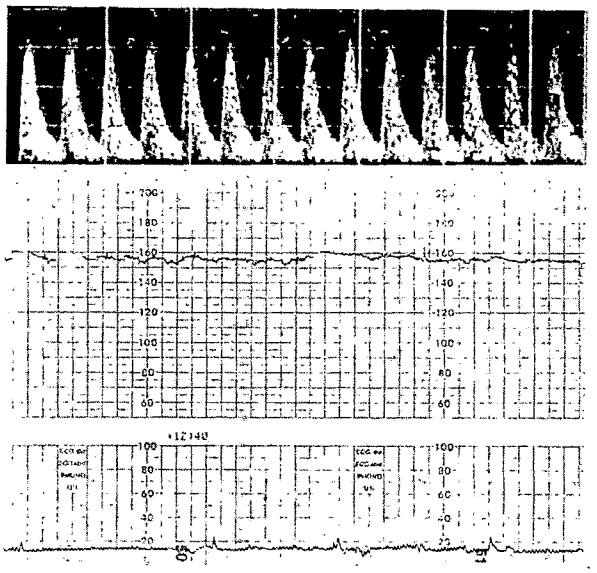


Fig. 5. Case No. 3, demonstrating a nonreactive nonstress test, moderately abnormal umbilical artery Doppler study (pH 7.34), and a good outcome.

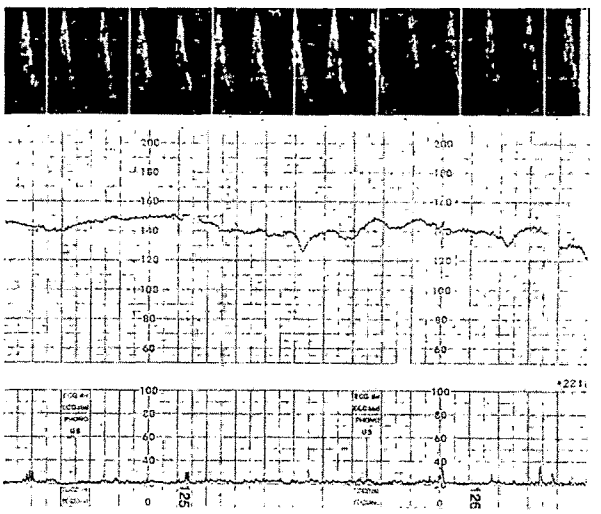


Fig. 6. Case No. 10, demonstrating a nonreactive nonstress test and abnormal umbilical artery Doppler study (pH 7.15) associated wtih birth asphyxia and neonatal death.

eight infants, six were in good condition at birth and two had only minor cord blood gas abnormalities. Figs. 5 and 6 demonstrate examples of nonreactive nonstress tests with poor variability, Doppler study results, and umbilical artery pH values. The nonstress test was nonreactive in both but there was a marked difference in the cord flow velocity waveform. The fetus with the higher pulsatility index value was severely compromised at birth.

Comment

Severe early onset growth retardation is relatively rare and it is difficult for one center to collect a large

series for study. In this small series, although 14 of 15 had abnormal umbilical artery flow velocity waveforms, the majority (nine) had a good outcome. When the pulsatility index was very abnormal, however, there was a highly significant risk of poor neonatal outcome, with five of six infants with pulsatility index values >2 having a poor outcome.

When an A/B ratio of greater than 100 was chosen (absent or reversed end-diastolic flow velocity), less accurate prediction of fetal risk was achieved inasmuch as three of these eight infants had a good outcome; however, there was still a significant association between high A/B ratio and poor outcome. This small difference between the two ratios in predicting outcome may arise because the pulsatility index differentiates between waveforms even when end-diastolic flow velocity is absent. This is because of an assessment of the area under the waveform, whereas the A/B ratio becomes infinity in all such cases.

Earlier studies of Erskine and Ritchie[8] and Trudinger et al.[10] demonstrated that an abnormal umbilical artery waveform was associated with a risk to the fetus but this risk could not be quantified, possibly because in most cases at least several days elapsed between Doppler study and delivery. In the present study all of the patients were examined on the day of delivery, usually only a few hours before birth.

The decision to deliver by cesarean section was independently made by the attending physician in the knowledge that the fetus was severely growth retarded and had a fetal heart tracing that was nonreactive or had poor variability. The interpretation of these tracings was complicated because five of the 11 nonreactive traces were in preeclamptic patients treated with magnesium sulfate, which is known to depress fetal heart rate variability.[4] Only three of the 11 had a low umbilical artery pH at birth. Aside from maternal medication, these results would support the work of Henson et al.[5] who found that a group of growth-retarded fetuses had significantly less fetal heart rate variation than control fetuses matched for gestational age and that such reduced variability was not necessarily related to fetal acidosis.

Our results will need to be confirmed in a larger series, but in the growth-retarded preterm fetus the degree of abnormality of the flow velocity waveform appears to be a more sensitive predictor of fetal outcome than a nonreactive nonstress test or a finding of reduced fetal heart rate variability.

In a woman with severe preeclampsia and a ruptured liver (Case No. 1), the fetus had a normal pulsatility index and A/B ratio. This is the first time we have seen a normal waveform in a severely growth-retarded preterm fetus and we are unable at present to explain this finding.

In four mothers there were no underlying maternal disorders. This group of patients is difficult to manage antenatally because one is always concerned about the risk of chromosomal or congenital anomalies or congenital viral infection. In this group two infants appear to be completely normal, another has trisomy 21, and one has a possible congenital viral infection. All four infants had very abnormal pulsatility index values; thus the pulsatility index was not helpful in differentiating between intrinsic and extrinsic causes of fetal growth retardation. This observation confirms that made previously by Trudinger and Cook.[11]

In summary, in the preterm growth-retarded fetus, umbilical artery flow velocity waveforms seem to carry information concerning fetal condition in utero that is more difficult to predict by other current methods such as the nonstress test. The technique, however, does not appear to allow differentiation between intrinsic and extrinsic causes of IUGR.

REFERENCES

1. Resnik R. Maternal diseases associated with abnormal fetal growth. J Reprod Med 1978;21:315.
2. Dawes G. Size at birth. In: Elliott K, Knight J, eds. Ciba Foundation Symposium, Amsterdam: Elsevier, 1974:393.
3. Keirse MJNC. Epidemiology and aetiology of the growth retarded baby. Clin Obstet Gynaecol 1984;11:415.
4. Lin CC, Pielet BW, Poon E, Sun G. The effect of magnesium sulfate on fetal heart rate variability in preeclamptic patients. In: Abstracts of fifth international congress of Society for the Study of Hypertension in Pregnancy. Nottingham, England: Society for the Study of Hypertension in Pregnancy, 1986.
5. Henson G, Dawes GS, Redman CWG. Characterization of the reduced heart rate variation in growth retarded fetuses. Br J Obstet Gynaecol 1984;91:751.
6. Fitzgerald DE, Drumm JE. Non-invasive measurement of the fetal circulation using ultrasound: a new method. Br Med J 1977;2:1450.
7. Olson RM, Cooke JP. Human carotid artery diameter and flow by a non-invasive technique. Med Instrum 1975;9:99.
8. Erskine RLA, Ritchie JWK. Umbilical blood flow characteristics in normal and growth retarded fetuses. Br J Obstet Gynaecol 1985;92:605.
9. Usher R, McLean F. Intrauterine growth of liveborn Caucasian infants at sea level. J Pediatr 1969;74:901.
10. Trudinger BJ, Giles WB, Cook CM, et al. Fetal umbilical artery flow velocity waveforms and placental resistance: clinical significance. Br J Obstet Gynaecol 1985;92:23.
11. Trudinger BJ, Cook CM. Umbilical and uterine artery flow velocity waveforms in pregnancy associated with major fetal abnormality. Br J Obstet Gynaecol 1985;92:666.

Adjuvant therapy for Stage I uterine sarcoma

Peter G. Rose, M.D., John G. Boutselis, M.D., and Larry Sachs, Ph.D.
Columbus, Ohio

A retrospective evaluation of adjuvant therapy in 64 patients with Stage I sarcoma was undertaken. A combination of operation and adjuvant radiation was compared with operation alone. A decreased recurrence of both pelvic and distant tumor was noted for endometrial sarcoma but not for leiomyosarcoma treated with adjuvant radiation. A Cox regression analysis showed a trend for improved survival, but the results were not statistically significant. Survival after vaginal cuff recurrence and treatment with radiation therapy (two patients) or combined radiation and chemotherapy (one patient) is reported. Seven patients received adjuvant chemotherapy with Adriamycin-based regimens. Chemotherapy alone did not statistically decrease recurrence in this small sample. (Am J Obstet Gynecol 1987;156:360-2.)

Key words: Uterine sarcoma, adjuvant therapy, radiation therapy, chemotherapy

Uterine sarcoma is rare, accounting for only 1% to 3% of uterine cancers. Its management is controversial and its rarity has hindered prospective studies. Because of the high recurrence rate of early stage disease (50% to 80%)[1-3] various adjuvant therapies have been used. Evaluations of these treatment regimens have relied extensively on retrospective studies that often span many years during which staging and treatment techniques have varied. We undertook such a retrospective study to determine whether adjuvant therapy affected the rate of recurrence in Stage I uterine sarcoma.

Methods

One hundred three patients with uterine sarcoma treated by The Ohio State University Gynecology Oncology Service from 1940 to 1984 were reviewed. At the time of diagnosis or surgical exploration, disease was not limited to the uterine corpus in 39 patients and they were excluded from this analysis. The remaining 64 patients were classified according to a modified uterine sarcoma nomenclature adopted by the gynecologic oncology group.[4] Patients were evaluated according to primary treatment regimen, and outcomes were statistically analyzed by the χ^2 test or Fisher's exact test.

Results

Sixty-four patients with Stage I uterine sarcoma included patients with carcinosarcoma (n = 17), endometrial stromal sarcoma (n = 10), fibrosarcoma (n = 1), leiomyosarcoma (n = 18), and mixed mesodermal sarcoma (n = 18). Patients were treated by operation (n = 32), radiation (n = 9), operation and radiation (n = 16), or operation and chemotherapy (n = 7). Adjuvant radiation treatment consisted of preoperative brachytherapy (n = 6), preoperative brachytherapy and teletherapy (n = 1), postoperative teletherapy (n = 7), and preoperative brachytherapy and postoperative teletherapy (n = 2). External radiation consisted of whole pelvic radiation, 4000 to 5000 rads in 200 rad fractions, over a period of 4 to 5 weeks. Brachytherapy consisted of a colpostat and tandem implant, delivering 3000 to 5000 rads to point A.

Chemotherapy consisted of Adriamycin-based regimens for 12 courses or until recurrence. Patients received vincristine, Adriamycin, and cyclophosphamide (VAC) (n = 4), cisplatin, Adriamycin, and cyclophosphamide (n = 1), Adriamycin and cyclophosphamide (n = 1), and Adriamycin, vincristine, and dacarbazine (DTIC) (n = 1).

In patients treated with operation or radiation alone, the recurrence rates were high, 75% and 77%, respectively. Treatment with operation and radiation or with operation and chemotherapy produced better results, with recurrence rates of 56% and 43%, respectively.

Statistical evaluation of treatment modality and subsequent recurrence was performed for all uterine sarcomas, endometrial sarcomas, and leiomyosarcomas. Recurrence of endometrial sarcoma was decreased for operation and radiation (five of 11) versus operation alone (22 of 27) ($p < 0.04$). The combination of operation and adjuvant chemotherapy was not statistically better than operation alone. Recurrence after radiation or operation alone (27 of 33) was significantly higher ($p < 0.05$) than after combined operation and adjuvant therapy (radiation or radiation and chemotherapy) (six of 13).

Addition of adjuvant treatment, radiation or che-

From the Department of Obstetrics and Gynecology and the Division of Research and Evaluation in Medical Education, The Ohio State University.
Received for publication July 1, 1986; accepted September 9, 1986.
Reprint requests: Dr. Peter G. Rose, 517 Means Hall, 1654 Upham Dr., Columbus, OH 43210.

motherapy, did not improve the survival or the time to recurrence for leiomyosarcoma.

When all uterine sarcomas were evaluated, improved outcome was seen only when combinations of operation and radiation or operation and chemotherapy were compared with operation or radiation alone ($p = 0.05$).

Despite decreased recurrence rates after adjuvant radiation therapy in patients with endometrial sarcoma, 45% had recurrent disease. The site of recurrence (pelvic versus distant) was not statistically different in patients treated with operation alone (45%) than in those treated by combined operation and radiation (40%). Radiation technique did not affect the recurrence rate in this series.

A Cox regression analysis of survival of patients with endometrial sarcoma, with correction for age at diagnosis and treatment at recurrence, showed a trend but no statistical improvement in this sample size.

Comment

The finding that the combination of operation and radiation is more effective than operation alone in preventing recurrent endometrial sarcoma but not leiomyosarcoma is in agreement with results of previous authors.

Belgrad et al.[5] reported on 34 patients, 16 of whom had Stage I disease. For all stages, radiation therapy resulted in an improved 2-year survival, except for those with leiomyosarcoma. Treatment results for patients with Stage I disease were not analyzed separately, however, and whether this radiation was adjuvant or therapeutic is unclear.

Gilbert et al.[6] analyzed 105 patients with uterine sarcoma, 57 of whom had Stage I disease. Although the authors favored adjuvant radiation therapy, only four patients with Stage I disease received primary operation and radiation therapy and no statistics were reported.

Vongtama et al.[7] studied 104 cases of Stage I or II uterine sarcoma and noted, in 87 patients with Stage I disease, a trend for improved survival with adjuvant radiation, but the results were not significant. In their series, pelvic radiation decreased local failure rates from 55% to 26%. No specific form of radiation therapy, whole pelvic or intracavitary, offered superior results.

Salazar et al.[8] reported on 18 patients with Stage I mixed mesodermal sarcoma. Of 12 patients treated with operation and radiation, only 50% had recurrences, in contrast to all six of the patients treated with operation alone ($p < 0.05$).

More recently, Sorbe[9] reported on 87 patients with uterine sarcoma, 59 of whom had Stage I (47) or II (12) disease. In 18 patients receiving adjuvant radiation therapy pelvic recurrences decreased, but there was no statistical improvement in survival.

Inasmuch as the addition of radiation in this study statistically decreased recurrence, both pelvic and distant, it would seem to be effective in treating local nodal disease and vaginal cuff recurrence before distant spread. Two patients in our study who had vaginal cuff recurrence were treated with external radiation and survived 20 and 206 months; one died of recurrent sarcoma and the other of a cerebrovascular accident. A third patient with recurrence that was limited to the vaginal cuff and treated with external radiation and chemotherapy (cyclophosphamide, vincristine, Adriamycin, and DTIC for 10 courses) is alive 52 months after recurrence. Buschbaum et al.[10] reported survival of a patient with mixed mesodermal sarcoma treated with radiation for vaginal cuff recurrence.

Because of the small number of patients treated prophylactically with chemotherapy, its role in Stage I uterine sarcoma is not defined by this study. In view of frequent distant recurrence and the proved although limited effectiveness of chemotherapy in advanced disease,[11, 12] there is great interest in prophylactic chemotherapy if Stage I disease exists. Buschbaum et al.,[9] reporting on 10 patients treated with VAC, noted an 80% 3- to 7-year survival. Two surviving patients had more than 10 mitoses per 10 high-power fields and no prior reports had documented survival in such cases. In nonrandomized studies, Hannigan et al.[13] reported no benefit of VAC chemotherapy and Barter et al.[14] reported no improvement with Adriamycin, VAC, or methotrexate, Adriamycin, and cyclophosphamide in a small series of patients. The gynecologic oncology group prospectively randomized 156 patients with Stage I or II uterine sarcoma treated with Adriamycin every 3 weeks for eight courses versus no additional therapy.[15] No improvement was noted for the Adriamycin-treated group but patients varied in their treatment with respect to adjuvant pelvic radiation.

The low response rate to chemotherapy in patients with established disease requires analysis of a large series of patients to prove that prophylactic therapy is beneficial. Whether the clinical benefit will outweigh the toxicity remains doubtful.

As with other malignancies, adequate staging of uterine sarcoma is important in determining appropriate therapy. More recently, the significance of pelvic (35%) and para-aortic (14.3%) lymph node metastasis in Stage I disease has been appreciated.[16] As with other sarcomas, hematogenous dissemination is rapid, with pulmonary metastasis the most common site of disease at autopsy.[17] Some authors advocate peritoneal cytologic washings and omentectomy as part of routine staging.[4] Although these pathologic variables are part of

our present staging protocol, they are not included in this series or in any other series in the literature.

A prospective study of postoperative pelvic radiation in Stage I and II mixed mesodermal sarcoma (Gynecologic Oncology Group 75-RTOG 8402) is in progress and is projected to be completed in 1994. The present data support adjuvant radiation for uterine sarcomas, except leiomyosarcomas, but only through prospective collaborative studies can our understanding and treatment of these rare malignancies be improved.

REFERENCES

1. Spanos WJ Jr, Peters LJ, Oswald MJ. Patterns of recurrence in malignant mixed müllerian tumor of the uterus. Cancer 1986;57:155-9.
2. Marchese MJ, Liskow AS, Crum CP, McCaffrey RM, Frick HC II. Uterine sarcomas: a clinicopathologic study, 1965-1981. Gynecol Oncol 1984;18:299-312.
3. Wheelock JB, Krebs HB, Schneider V, Goplerud DR. Uterine sarcoma: analysis of prognostic variables in 71 cases. Am J Obstet Gynecol 1985;151:1016-22.
4. Lotocki RJ. Can we cure mixed müllerian tumors? Contemp Ob Gyn 1984;23:155-78.
5. Belgrad R, Elbadawi N, Rubin P. Uterine sarcoma. Radiology 1975;114:181-8.
6. Gilbert HA, Kagan AR, Lagasse L, Jacobs MR, Tawa K. The value of radiation therapy in uterine sarcoma. Obstet Gynecol 1975;45:84-8.
7. Vongtama V, Karlan JR, Piver SM, Tsukada Y, Moore RH. Treatment, results and prognostic factors in stage I and II sarcomas of the corpus uteri. Am J Roentgenol 1976;126:139-47.
8. Salazar OM, Bonfiglio TA, Patten SF, Keller BE, Feldstein ML, Dunne ME, Rudolph JH. Uterine sarcomas: analysis of failures with special emphasis on the use of adjuvant radiation therapy. Cancer 1978;42:1161-70.
9. Sorbe B. Radiotherapy and/or chemotherapy as adjuvant treatment of uterine sarcomas. Gynecol Oncol 1985;20:281-9.
10. Buchsbaum HJ, Lifshitz S, Blythe JG. Prophylactic chemotherapy in stages I and II uterine sarcoma. Gynecol Oncol 1979;8:346-8.
11. Omura GA, Major FJ, Blessing JA, et al. A randomized study of Adriamycin with and without dimethyl triazenoimidazole carboxamide in advanced uterine sarcoma. Cancer 1983;52:626-32.
12. Hannigan EV, Freedman RS, Elder KW, Rutledge FN. Treatment of advanced uterine sarcoma with vincristine, actinomycin D and cyclophosphamide. Gynecol Oncol 1983;15:224-9.
13. Hannigan EV, Freedman RS, Rutledge FN. Adjuvant chemotherapy in early uterine sarcoma. Gynecol Oncol 1983;15:56-64.
14. Barter FJ, Smith EB, Szpak CA, Hinshaw W, Clarke-Pearson DL, Creasman WT. Leiomyosarcoma of the uterus: clinicopathologic study of 21 cases. Gynecol Oncol 1985;21:220-7.
15. Omura GA, Blessing JA, Major F, et al. A randomized clinical trial of adjuvant Adriamycin in uterine sarcomas: a gynecologic oncology group study. J Clin Oncol 1985;3:1240-45.
16. DiSaia PJ, Morrow CP, Boronow R, Creasman W, Mittelstaedt L. Endometrial sarcoma: lymphatic spread pattern. Am J Obstet Gynecol 1978;130:104-5.
17. Fleming WP, Peters WA, Kumar NB, Morley GW. Autopsy findings in patients with uterine sarcoma. Gynecol Oncol 1984;19:168-72.

Clinicopathologic study of eight cases of uterine body cancers associated with endometriosis interna (uterine adenomyosis)

Takashi Hayata, M.D., and Yoshiro Kawashima, M.D.
Hamamatsu, Japan

Among 30 cases of uterine body cancers, in eight cases (Stage IA, two cases; Stage IB, six cases) uterine adenomyosis was demonstrated microscopically. The age range was from 46 to 66 years with a median of 56. When these eight cases were compared with the 12 cases of Stage I endometrial cancer without adenomyosis, there was no difference in either menstrual history or family history, although past histories of hypertension and diabetes mellitus were found in these eight cases. The mean obesity index was 127 in eight cases and 116 in 12 cases. Seven of these eight cases were pure tubular adenocarcinoma. From the standpoint of early myometrial infiltration of the endometrial cancer, these eight cases not only provided a good model to survey early endometrial cancer but also suggested a common stimulus, such as estrogen, in both endometrial cancer and uterine adenomyosis. (Am J Obstet Gynecol 1987;156: 663-6.)

Key words: Endometrial cancer, adenomyosis, clinicopathologic study

Endometriosis interna (adenomyosis), which is very painful in menstruating women and results in severe dysmenorrhea, was studied clinicoendocrinologically. In a study of 30 cases of endometrial cancer at our hospital, we found uterine adenomyosis in specimens from eight of the 20 cases of early endometrial cancer. These uterine cancers that contained microscopic adenomyosis were studied clinicopathologically and were compared with 12 cases of endometrial cancer without adenomyosis.

Material and methods

Details of eight cases of endometrial cancer with adenomyosis are shown in Table I. The age range was 46 to 66 years, with a median of 56. Parity and gravidity averaged four and two, respectively. The chief complaint in five of the eight cases was genital bleeding. All cancers were classified as Stage I according to the criteria of the International Federation of Gynecology and Obstetrics (two were Stage IA and six were IB).

Results

Menstrual history. There were no significant findings in regard to age at menarche or at marriage. Menstrual cycles were regular in four cases and irregular in four cases. One patient with regular menses and two with irregular menses had dysmenorrhea. Age at menopause in five cases ranged from 45 to 53; the other three patients were premenopausal (Table II). The duration from menarche to menopause, to last delivery (or pregnancy), and to operation is shown in Table III. When one shows the time to menopause as ~, the time from menarche to menopause or the duration of menstruation ranges from 28 to ~41 years (average of ~36). The period between menopause and operation ranged from 2 to 15 years (average of 8 years). The period from last delivery (or pregnancy) to operation was between 23 (17 for pregnancy) to 35 (33 for pregnancy) [average of 27 (or 23 for pregnancies)].

Medical history (Table IV). In three of the eight cases hypertension was an associated finding and there was one case of diabetes mellitus. In the other 12 cases of Stage I uterine cancer and without adenomyosis, hypertension and diabetes mellitus were not found.

Family history (Table V). In two of the eight cases, a father and an elder brother had diabetes mellitus. In five cases first- or second-pedigree relatives have died of cancer. On the contrary, in the 12 cases of endometrial cancer without adenomyosis, there were no cases of diabetes mellitus or cancer elsewhere in the body.

Obesity index (Table VI). The obesity index was calculated by the formula: body weight × 100 divided by (height − 100) × 0.9. The obesity index score in eight cases of Stage I uterine corpus cancer with adenomyosis ranged from 100 to 147 (average = 127). In 12 cases of Stage I uterine corpus cancer without adenomyosis the average score was 116. In 30 cases of Stage I cervical cancer with a similar age range the obesity index score was 106.

Histopathologic findings (Table VII). The histologic

From the Department of Obstetrics and Gynecology, Hamamatsu University School of Medicine.
Presented at the Eleventh World Congress of Gynecology and Obstetrics, West Berlin, September 15-20, 1985.
Received for publication March 6, 1986; revised June 16, 1986; accepted July 12, 1986.
Reprint requests: Takashi Hayata, M.D., Department of Obstetrics and Gynecology, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu, 431-31, Japan.

Table I. Details of eight cases of uterine corpus cancer with adenomyosis

Case No.	*Age (yr)*	*Gravidity*	*Parity*	*Chief complaint*	*Stage*	*Menopausal status*
1	46	4	2	Hypermenorrhea-polymenorrhea	IB	Premenopausal
2	55	3	2	Genital bleeding	IA	Postmenopausal
3	48	6	3	Genital bleeding	IB	Premenopausal
4	66	6	4	Genital bleeding	IB	Postmenopausal
5	60	5	3	Genital bleeding	IA	Postmenopausal
6	54	1	0	Genital bleeding	IB	Postmenopausal
7	56	1	0	Lower abdominal pain	IB	Premenopausal
8	59	7	5	Cancer check	IB	Postmenopausal
Mean	56	4	2			

Table II. Menstrual history

Case No.	*Age (yr)*	*Age at menarche (yr)*	*Age at marriage (yr)*	*Menstrual cycle regularity*	*Menstrual symptoms*	*Age at menopause (yr)*
1	46	15	20	Irregular	Dysmenorrhea	—
2	55	13	22	Regular	—	53
3	48	14	24	Irregular	—	—
4	66	15	22	Regular	Dysmenorrhea	53
5	60	17	21	Irregular	—	45
6	54	15	30	Regular	—	52
7	56	15	31	Irregular	Dysmenorrhea	—
8	59	12	18	Regular	—	51

Table III. Reproductive history

Case No.	*Age at present (yr)*	*Age at menarche (yr)*	*No. of reproductive years*	*Age at menopause (yr)*	*No. of years since menopause*	*No. of years since last delivery*	*No. of years since last pregnancy*
1	46	15	>31	—		23	17
2	55	13	40	53	2	27	
3	48	14	>34	—		23	18
4	66	15	38	53	13	35	33
5	60	17	28	45	15	27	
6	54	15	37	52	2		25
7	56	15	>41	—			24
8	59	12	39	51	8	29	

Table IV. Medical history

Case No.	*Age at present (yr)*	*History*	*Age at onset (yr)*	*Duration*
1	46	Abdominal pain	35	—
2	55	Left ovarian cystectomy	38	
		Hypertension (drug treatment)	~53	2 yr
3	48	Hypertension	~43	5 yr
		Hepatitis B surface and e antigen positive	~46	2 yr
4	66	Diabetes mellitus, hypertension	~50	16 yr
		Angina pectoris	~55	11 yr
		Gallstones	59	—
		Senile colpitis	66	1½ mo
5	60	Senile colpitis	60	2 wk
		Constipation	60	—
6	54	Acute hepatitis	29	—
		Appendectomy	30	—
		Left ventricular hypertrophy	~50	4 yr
7	56	Pleurisy	23	—
8	59	Heart disease	~50	9 yr

Table V. Family history

Case No.	Relative	History
1	Father	Diabetes mellitus
4	Grandfather	Rectal polyp
	Mother of grandmother	Cervical cancer*
5	Father	Stomach cancer*
	Mother	Rectal (?) cancer*
7	Grandfather	Stomach cancer*
	Younger brother	Stomach cancer*
	Younger sister	Uterine myoma (removed)
8	Aunt	Rectal cancer*
	Elder brother	Diabetes mellitus

*Deceased.

Table VI. Obesity index

Case No.	Height (cm)	Weight (kg)	Obesity index score
1	141	44	119
2	146	55	133
3	143	55	142
4	147	57	135
5	150	45	100
6	157	68	133
7	150	66	147
8	155	51	103
Mean	149	55	127

Obesity index calculated as: $\frac{\text{Weight} \times 100}{(\text{Height} - 100) \times 0.9}$

Table VII. Histologic types of carcinoma

Case No.	Age (yr)	Type of cancer	Endometrial background	Adenomyotic glandular tissue
1	46	Tubular adenocarcinoma	Proliferative endometrium	Proliferative endometrium-like
2	55	Tubular (comedo-like) adenocarcinoma	Proliferative endometrium	Proliferative endometrium-like
3	48	Tubular adenocarcinoma	Atrophy	Proliferative endometrium-like
4	66	Papillotubular adenocarcinoma	Day 20 endometrium	Day 19 endometrium-like
5	60	Tubular adenocarcinoma	Proliferative endometrium	Proliferative endometrium-like
6	54	Tubular adenocarcinoma	Proliferative endometrium with focal cystic glandular hyperplasia	Proliferative endometrium-like
7	56	Tubular adenocarcinoma	Day 27 endometrium	Day 23 endometrium-like
8	59	Tubular adenocarcinoma	Proliferative endometrium	Proliferative endometrium-like

type of the presented cases of corpus cancer was well-differentiated tubular adenocarcinoma with the exception of papillotubular adenocarcinoma in case No. 4. The histologic features that preceded these corpus cancers were proliferative endometrium in five cases, atrophy in one, and secretory endometrium in two. Adenomyotic glandular epithelium demonstrated a proliferative endometrium–like pattern, except in two cases with secretory endometrium–like adenomyotic glandular tissue. Histologic examination of the ovaries revealed five cases with a corpus albicans and two with a corpus luteum. There was one case of ovarian endometriosis (Case No. 1) without any malignant transformation pattern.

Comment

More cases of microscopic adenomyosis in cases of uterine corpus cancer have been reported than one might estimate. Giammalvo and Kaplan[1] stated that among 120 cases of endometrial cancer (all were adenocarcinomas, with exclusion of carcinosarcomas, autopsy cases, and cases without a description of the clinical stages), 40 (33%) contained microscopic adenomyosis. Marcus[2] found 60 cases of adenomyosis in 100 endometrial cancers in comparison with 39 in 100 cases in the control group. In 39 of 60 cases of adenomyosis associated with endometrial cancer, endometrial hyperplasia was also demonstrated, which is significantly different from the seven among 39 cases in the control group.

The fact that eight of our own 30 cases of endometrial cancer associated with adenomyosis were all Stage I leads us to consider the possibility that these cases could be good models in which to follow the myometrial infiltration of corpus cancer in its early phase.

Endometrial hyperplasia as a histologic background of corpus cancer, such as in those cases in the report of Marcus,[2] was not found, except for the one case with focal cystic glandular hyperplasia. In other words examination of the endometrium before the cancer was diagnosed in seven of eight cases revealed normal histologic features.

Our own 100 cases of adenomyosis (microscopic or clinical) up to the present have shown no instances of malignant transformation of the adenomyotic glandular epithelium histologically.

That two of the eight cases described here had atypical glandular epithelium (a definite diagnosis such as adenomyotic glandular tissue was not possible because of the lack of endometrial stroma) suggests the possibility of an early myometrial infiltrating pattern of endometrial cancer.

In any case data such as histologic findings and clinical history (menstrual history, medical history, family

history, and obesity index score) suggest that some common stimuli such as estrogen promote both uterine corpus cancer and adenomyosis.

REFERENCES

1. Giammalvo JT, Kaplan K. The incidence of endometriosis interna in 120 cases of carcinoma of the endometrium. AM J OBSTET GYNECOL 1958;75:161.
2. Marcus CC. Relationship of adenomyosis uteri to endometrial hyperplasia and endometrial carcinoma. AM J OBSTET GYNECOL 1961;82:408.

Type-specific streptococcal antibodies in amniotic fluid

Barry M. Gray, M.D., Janet D. Springfield, M.P.H., and Hugh C. Dillon, Jr., M.D.
Birmingham, Alabama

Type-specific antibodies against group B streptococcal and pneumococcal capsular antigens were measured in 68 amniotic fluid samples from healthy women between 16 and 20 weeks of pregnancy. Mean antibody levels ranged from 0.13 to 0.61 μg/ml, with few samples having >1 μg/ml against any antigen. The role of antibodies in protection against intrauterine infection is discussed. (AM J OBSTET GYNECOL 1987;156:666-9.)

Key words: Group B streptococci, pneumococci, antibodies, amniotic fluid

Amniotic fluid normally contains low but measurable amounts of immunoglobulins, mostly of the IgG class.[1] Immunoglobulin levels of all classes are fairly constant throughout gestation, with a decrease in IgG at term, probably because of changes in amniotic fluid volume.[2] The role of specific antibodies in amniotic fluid has yet to be explored, although there is some evidence for both specific and nonspecific opsonization of *Escherichia coli* and group B streptococci by term and preterm amniotic fluid.[3] In this study we offer quantitative data on amniotic fluid levels of type-specific antibody against the four major polysaccharide antigens of the group B streptococci and eight common capsular types of *Streptococcus pneumoniae.*

Methods

Amniotic fluid samples were collected from women attending the Prenatal Genetics Clinic of the University of Alabama at Birmingham in December 1984, according to methods previously described.[4] Indications for amniocentesis included maternal age ≥35 years, family history of congenital anomalies, and history of obstetric complications. No cultures for carriage of group B streptococci or pneumococci were done, and no sera were available for study. Sixty-eight clear fluid samples were selected randomly from otherwise healthy women between 16 and 20 weeks of pregnancy. The amniotic fluid specimens were initially processed in the Laboratory of Medical Genetics; the centrifuged supernatant fluids were stored at −20° C until used for antibody determinations.

Antibodies to group B streptococci and pneumococcal polysaccharide antigens were measured quantitatively by enzyme-linked immunoassay methods modified from those described for group B streptococci type II.[5] Group B streptococci type Ib, II, and III polysaccharides were prepared by mutanolysin extraction and purified by chromatography, essentially as described by De Cueninck et al.[6] The type Ia polysaccharide was prepared by sonication in cold 2.5% trichloroacetic acid followed by separation of group and type antigens over a Sepharose 6B column.[7] Purified pneumococcal polysaccharides of types 1, 3, 4, 6, 9, 14, 19, and 23 were obtained from Lederle Laboratories, Pearl River, New York, and from Eli Lilly & Company, Indianapolis, Indiana. Each polysaccharide was coupled to polylysine[8] and dispensed in 300 μl volumes into plastic microtiter wells (Dynatech Laboratories, Alexandria, Virginia). The wells were incubated overnight at room

From the Department of Pediatrics, University of Alabama at Birmingham.
This work was supported by National Institute of Child Health and Human Development Program Project Grant No. 1-PO5-HD-17812.
Received for publication March 11, 1986; revised September 23, 1986; accepted October 17, 1986.
Reprint requests: Dr. Barry M. Gray, Department of Pediatrics, University of Alabama at Birmingham, University Station, Birmingham, AL 35294.

Table I. Antibody levels in 68 amniotic fluid samples

	Amniotic fluid antibody (μg/ml)			
	Mean	*1 SD*	*Highest value*	*No. with >1 μg/ml*
Group B streptococci				
Type Ia	0.18	0.44	3.55	2
Type Ib	0.35	0.26	1.02	1
Type II	0.40	0.44	1.64	8
Type III	0.29	0.33	1.73	3
Streptococcus pneumoniae				
Type 1	0.15	0.13	0.69	0
Type 3	0.13	0.10	0.40	0
Type 4	0.61	0.51	2.06	7
Type 6	0.19	0.22	1.23	2
Type 9	0.15	0.12	0.45	0
Type 14	0.53	0.50	2.62	8
Type 19	0.34	0.46	2.86	5
Type 23	0.15	0.15	0.83	0

temperature, washed with deionized water, filled with 0.1% bovine serum albumin, and refrigerated until use.

Amniotic fluid was added to the wells in 50 μl volumes and diluted with 250 μl of phosphate-buffered saline–Brij (0.05 mol/L phosphate, 0.1 mol/L sodium chloride, and 0.05% Brij, Aldrich Chemical, Atlanta, Georgia), pH 7.4. After incubation for 3 hours at room temperature, unbound antibody was washed out with deionized water. β-Galactosidase conjugated goat antihuman $F(ab')_2$ (Southern Biotechnology Associates, Inc., Birmingham, Alabama) was diluted 1:500 in a phosphate-buffered saline–Brij buffer of greater ionic strength (0.55 mol/L sodium chloride and 0.05 mol/L phosphate, pH 7.4) and added to the wells in 300 μl volumes. After 2 hours of incubation the wells were again washed, and 300 μl of enzyme substrate was added to each well. The substrate was o-nitrophenyl-β-D-galactopyranoside (Sigma Chemical Company, St. Louis, Missouri) diluted to 1 mg/ml in 0.01 mol/L Tris, pH 7.5, containing 0.01 mol/L concentrations of sodium chloride, magnesium chloride, and 2-mercaptoethanol. The change in optical density was read after 20 minutes at 414 nm with an eight-channel photometer (Titertek Multiskan, Flow Laboratories, McLean, Virginia). Results, expressed as absorbance units, were converted to micrograms of antibody protein per milliliter by calculation from a standard curve consisting of IgG measured by quantitative precipitation for antibody against a pneumococcus type 19 antigen.[5] Although the anti-$F(ab')_2$ conjugate detected antibodies of all classes, most (80%) amniotic fluid antibody at 16 to 20 weeks' gestation is of the IgG class[1] and values were assumed to be equivalent in micrograms to the amount of IgG in the standard. The linear portion of the curve corresponded to 0.03 to 1.42 μg/ml of amniotic fluid, on the basis of 50 μl samples. Further sample dilutions were made as necessary, and all assays were done in duplicate.

Results

Antibody levels in 68 amniotic fluid samples are presented in Table I. Mean levels were 0.18 to 0.40 μg/ml against group B streptococcal antigens; the highest levels were against type II, followed by types Ib, III, and Ia. Antibodies against pneumococcal polysaccharides averaged 0.13 to 0.61 μg/ml, the highest being those for types 4 and 14. Levels ranged as high as 1 to 3 μg/ml, but very few samples had >1 μg/ml.

It was of some interest to note that within a given patient there was a tendency to have little or no antibody against any group B streptococcal type or to have antibodies against multiple types. This is illustrated in Fig. 1, counting the number of types for which a patient had greater than the mean antibody level. The distribution was far more uniform (Pearson χ^2 (4 df) = 31.8, $p < 0.0001$). The 31/68 patients who had no antibody greater than the mean accounted for most of the deviation (their contribution to the total χ^2 = 22.3), while the frequency of those with antibody against two or three types was lower than expected. No such striking distribution was seen with respect to antibodies against pneumococcal polysaccharides.

Comment

Amniotic fluid appears to play a role in protecting the fetus from bacterial infection by a variety of mechanisms.[9] Certain organisms, including *Escherichia coli*, may be inhibited by a zinc-dependent peptide[9] in the absence of complement and phagocytes. The group B streptococci are inhibited little or not at all by amniotic fluid alone,[10-12] but specific as well as nonspecific opsonic activity has been demonstrated in a chemiluminescence assay in the presence of human peripheral neutrophils.[3] Unlike gram-negative organisms, which may be lysed by antibody and complement activation, the group B streptococci require antibody, complement, and phagocytosis to effect killing.[13]

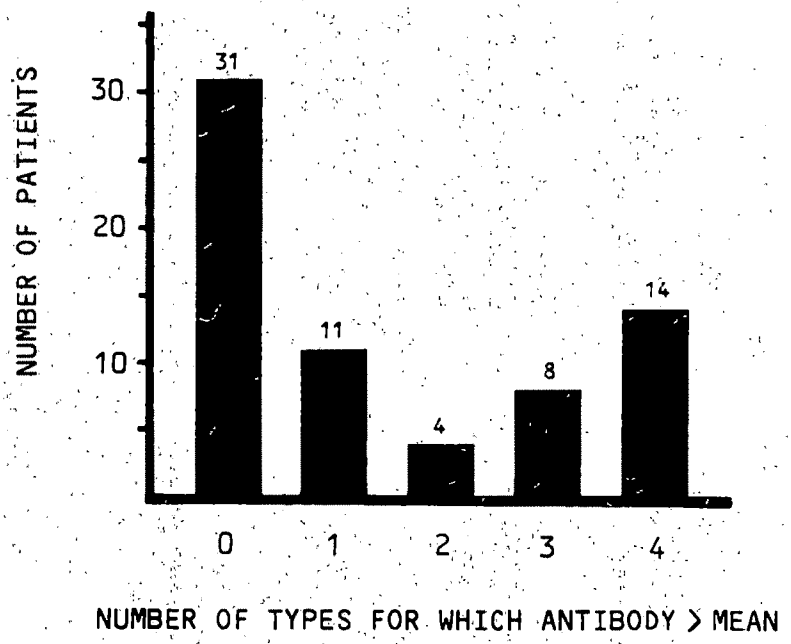


Fig. 1. Sixty-eight amniotic fluid samples were tested for antibody against group B streptococci types Ia, Ib, II, and III. The figure shows the distribution of patients according to the number of types for which the antibody level was greater than the mean. The proportion of patients with antibody greater than the mean against four types or against no types was greater than expected (see Results). No such type-related distribution was seen for antibody against the eight pneumococcal types tested.

In previous studies we found the prevalence of serum IgG against group B streptococci types Ia, II, and III quite low, with only about 6% of women at delivery having >2 μg/ml.[5, 14] Carriers of group B streptococci were more likely to have antibody against one or more types, whereas noncarriers rarely had antibody. Although culture data were not available from patients in the present study, antibody levels were less than the mean for all four types in nearly half the specimens. To get a broader picture of antibody prevalence against gram-positive capsular polysaccharides, we also measured levels against common pneumococcal types. The rarity of amniotic infection due to pneumococci is not likely to be explained by the presence of antibody, which was as low as that against group B streptococci; rather it probably reflects differences in epidemiology of this respiratory pathogen. The presence of antibody to group B streptococci, in serum or in amniotic fluid, may have a protective role, but the vast majority of individuals, with little or no antibody, are at no apparent risk for perinatal disease. On the other hand, antibody present at the time of infection may not always be adequate to forestall invasion.[5]

It is nonetheless quite reasonable to propose that the presence in amniotic fluid of even small amounts of type-specific antibody may be beneficial. Unless rupture of the membranes has occurred, the numbers of group B streptococci gaining access to the amniotic cavity are likely to be very small. An average antibody concentration of 0.4 μg/ml multiplied by 500 to 1000 ml of fluid is a considerable amount. Complement may be available in amniotic fluid and certainly is available in perfused tissues. Neutrophils are not a regular constituent of amniotic fluid but are often present as the result of a maternal inflammatory response.[15] Intrauterine invasion by bacteria may be hindered enough to prevent infection of the fetus. This is consistent with the observation that infants delivered of mothers with group B streptococcal amnionitis may escape infection even though they are heavily colonized at birth.[16] These hypotheses remain to be tested, and other explanations have yet to be explored.

We wish to thank Drs. Wayne and Sara Finley and the staff of the Laboratory of Medical Genetics and Drs. Richard Davis and Cynthia Brumfield of the Department of Obstetrics and Gynecology. Dr. David B. Pritchard provided purified group B streptococcal polysaccharides.

REFERENCES

1. Cederqvist LL, Ewool LC, Bonsnes RW, Litwin SD. Detectability and pattern of immunoglobulins in normal amniotic fluid throughout gestation. Am J Obstet Gynecol 1978;130:220-4.
2. Blanco JD, Gibbs RS, Krebs LF. A controlled study of amniotic fluid immunoglobulin levels in intraamniotic infection. Obstet Gynecol 1983;61:450-3.

3. Cone MJ, Steele RW, Marmer DJ, Hill DE. Functional bacterial opsonic activity of human amniotic fluid. Am J Obstet Gynecol 1982;142:282-7.
4. Cassell GH, David RO, Waites KB, Brown MB, Marriott PA, Stagno S, Davis JK. Isolation of *Mycoplasma hominis* and *Ureaplasma urealyticum* from amniotic fluid at 16-20 weeks of gestation: potential effect on outcome of pregnancy. Sex Trans Dis 1983;10(suppl):294-302.
5. Gray BM, Pritchard DG, Dillon HC Jr. Seroepidemiologic studies of group B streptococcus type II. J Infect Dis 1985;151:1073-80.
6. De Cueninck BJ, Shockman GD, Swenson RM. Group B, type III streptococcal cell wall: composition and structural aspects revealed through endo-N-acetylmuramidase-catalyzed hydrolysis. Infect Immun 1982;35:572-82.
7. Gray BM, Dillon HC Jr, Pritchard DG. Interaction of group B streptococcal type-specific polysaccharides with wheat germ agglutinin and other lectins. J Immunol Methods 1984;72:269-77.
8. Gray BM. ELISA methodology for polysaccharide antigens: protein coupling of polysaccharides for adsorption to plastic tubes. J Immunol Methods 1979;28:187-92.
9. Schlievert P, Johnson W, Galask RP. Amniotic fluid antibacterial mechanisms: newer concepts. Semin Perinatol 1977;1:59-70.
10. Appelbaum PC, Shulman G, Chambers NL, et al. Studies on the growth-inhibiting property of amniotic fluids from two United States population groups. Am J Obstet Gynecol 1980;137:579-82.
11. Blanco JD, Gibbs RS, Krebs LF. Inhibition of group B streptococci by amniotic fluid from patients with intraamniotic infection and from control subjects. Am J Obstet Gynecol 1983;147:247-50.
12. Hemming VG, London WT, Nagarajan K, et al. Failure of inhibition of in vivo group B streptococcus growth in rhesus amniotic fluid. Am J Obstet Gynecol 1984;149:230-1.
13. Shigeoka AO, Hall RT, Hemming VG, Allred CD, Hill HR. Role of antibody and complement in opsonization of group B streptococci. Infect Immun 1978;21:34-40.
14. Gray BM, Dillon HC Jr. Prevalence of antibody to GBS types Ia, II and III [Abstract 238]. In: Program and abstracts of the twenty-fourth interscience conference on antimicrobial agents and chemotherapy. Washington DC: American Society for Microbiology, 1984:132.
15. Vasan U, Lim DM, Greenstein RM, Raye JR. Origin of gastric aspirate polymorphonuclear leukocytes in infants born after prolonged rupture of membranes. J Pediatr 1977;91:69-72.
16. Gray BM, Dillon HC Jr. GBS infections in mothers and their infants. Antibiot Chemother 1985;35:225-36.

Luteal function in ectopic pregnancy

Corinne J. Hubinont, M.D., Christine Thomas, M.D., and Jean F. Schwers, M.D.
Brussels, Belgium

Human chorionic gonadotropin, estradiol, progesterone, and 17-hydroxyprogesterone have been measured in the serum of 46 patients with ectopic pregnancy. All these hormones were significantly lower than in normal pregnancy. Unruptured ectopic pregnancy differed from the ruptured state by a lower serum human chorionic gonadotropin concentration, a slower human chorionic gonadotropin increment, a higher incidence of metrorrhagia, and an earlier diagnosis. The concentration of estradiol, progesterone, and 17-hydroxyprogesterone in the serum of patients with ectopic pregnancy was lower than could be expected from the decrease of human chorionic gonadotropin, often lower than in a normal luteal phase. It is suggested that, as long as ultrasonography fails to show an intrauterine pregnancy, the simultaneous determination of serum human chorionic gonadotropin and progesterone could aid in the early diagnosis of ectopic pregnancy and in the improvement of subsequent fertility; to that regard any progesterone level below 15 ng/ml in the presence of detectable amounts of human chorionic gonadotropin is highly suggestive of either a threatened abortion or an ectopic pregnancy, whatever the gestational age. (Am J Obstet Gynecol 1987;156:669-74.)

Key words: Ectopic pregnancy, corpus luteum, human chorionic gonadotropin, estradiol, progesterone, 17-hydroxyprogesterone

The low level of serum human chorionic gonadotropin in patients with ectopic pregnancy is well documented, but very little attention has been paid to the evaluation of luteal function in these patients. It is now established that the serum human chorionic gonadotropin concentration is significantly reduced in cases of ectopic pregnancy[1-4] and that repeated human chorionic gonadotropin determinations show a very slow increase in ectopic pregnancy as compared with normal pregnancy.[5,6] As regards the luteal function it has only been mentioned by Milwidsky et al.[7] that the ser-

From the Department of Obstetrics and Gynecology, Erasme Hospital, Brussels University.
Received for publication November 5, 1985; revised July 29, 1986; accepted September 26, 1986.
Reprint requests: Dr. Corinne J. Hubinont, Department of Obstetrics and Gynecology, Hopital Erasme, Route de Lennik, 808, 1070 Brussels, Belgium.

Table I. Mean human chorionic gonadotropin concentration in the sera of patients with unruptured and ruptured ectopic pregnancy and with normal pregnancy

Gestational age (wk)*	*Human chorionic gonadotropin (ng/ml)*		
	Ectopic pregnancy		*Normal pregnancy*
	Unruptured	*Ruptured*	
6	113 (n = 5)	228† (n = 5)	4,580 (n = 40)
7	217 (n = 10)	1452† (n = 11)	8,200 (n = 29)
8	183 (n = 6)	857† (n = 7)	11,820 (n = 29)

Number of samples is shown in parentheses.
*Completed weeks from last menstrual period.
†$p < 0.001$ for t test between ruptured and unruptured ectopic pregnancies.

um progesterone level is lower than that in normal pregnancy.

Some investigators[6] have shown that repeated human chorionic gonadotropin determinations would be helpful in cases of suspected ectopic pregnancy, but no one has ever suggested that the simultaneous determination of human chorionic gonadotropin and ovarian steroids could also provide some useful information when ultrasonography fails to demonstrate an intrauterine pregnancy.

In the present investigation, human chorionic gonadotropin, estradiol, progesterone, and 17-hydroxyprogesterone have been measured in the serum of 46 patients with ectopic pregnancy and compared with normal values. The differences between ruptured and unruptured ectopic pregnancy were also analyzed as regards to hormone levels and incidence of uterine bleeding.

Material and methods

Forty-nine consecutive and pathologically verified ectopic pregnancies were studied retrospectively. All blood samples had been collected beween 3 and 10 weeks and always before operation. Three ovarian pregnancies were excluded from the present study; of the remaining 46 tubal pregnancies 18 (39%) were ruptured at the time of operation. One to five blood samples were collected from each patient. Serum human chorionic gonadotropin, estradiol, progesterone, and 17-hydroxyprogesterone were measured by specific radioimmunoassays.

The normal range for each of these hormones was previously determined from some 800 serum samples collected between 3 and 42 weeks of gestation (nearly 200 between 3 and 10 weeks) and processed by the same radioimmunoassay. The gestational age is expressed as completed weeks from last menstrual period.

Since the data from hormone assays usually show a log normal distribution rather than a normal distribution, statistical parameters such as mean, SD, t test, and correlations were calculated on a logarithmic basis. The lower and upper limits of the normal range were set at the tenth and ninetieth percentiles, respectively. The χ^2 tests were performed with the use of the correction for small numbers.[8]

Results

Human chorionic gonadotropin. Nearly all human chorionic gonadotropin values fell below the normal range, except for the samples collected during the fourth week of pregnancy. As shown in Table I, mean human chorionic gonadotropin levels were significantly lower in unruptured than in ruptured ectopic pregnancies ($p < 0.001$). The human chorionic gonadotropin slope from 5 to 9 weeks also showed a difference between these two groups, with a mean value of 23 ng/day for the former and 43 ng/day for the latter. In normal pregnancy the mean increase of human chorionic gonadotropin during that period of gestation is linear and reaches 500 ng/day.

Steroids. Levels of ovarian steroids were generally below the normal range (Figs. 1 to 3) but the difference between ruptured and unruptured ectopic pregnancies was not as evident as for human chorionic gonadotropin. For the samples collected at 7 and 8 weeks a significant positive correlation exists between human chorionic gonadotropin and progesterone ($r = 0.6384$, $p < 0.01$ for 20 df) and between human chorionic gonadotropin and estradiol ($r = 0.4469$, $p < 0.05$ for 18 df) but not between human chorionic gonadotropin and 17-hydroxyprogesterone.

Although a significant correlation did exist between human chorionic gonadotropin and progesterone the decrease of the latter was apparently more important than that of the former; in other words the progesterone concentration in ectopic pregnancy seemed lower than that expected in a normal pregnancy with a similar human chorionic gonadotropin level. In order to assess

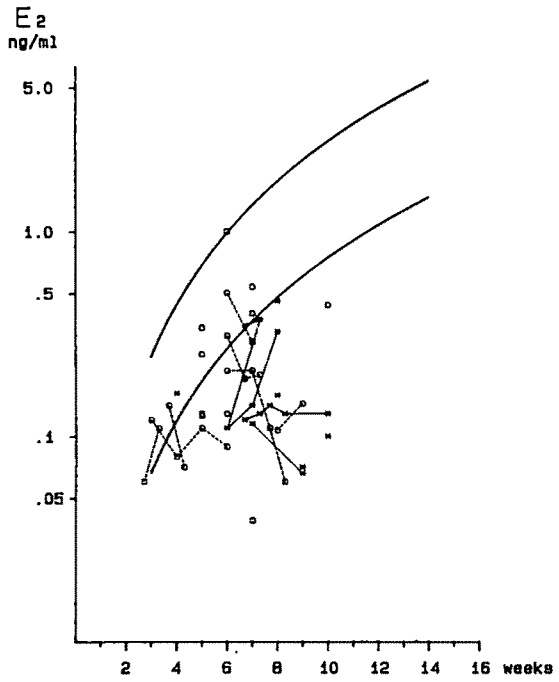


Fig. 1. Serum estradiol *(E_2)* concentration in patients with ectopic pregnancy (o for unruptured ectopic pregnancies and * for ruptured ectopic pregnancies). The *solid lines* represent the limits of the normal range (tenth and ninetieth percentiles).

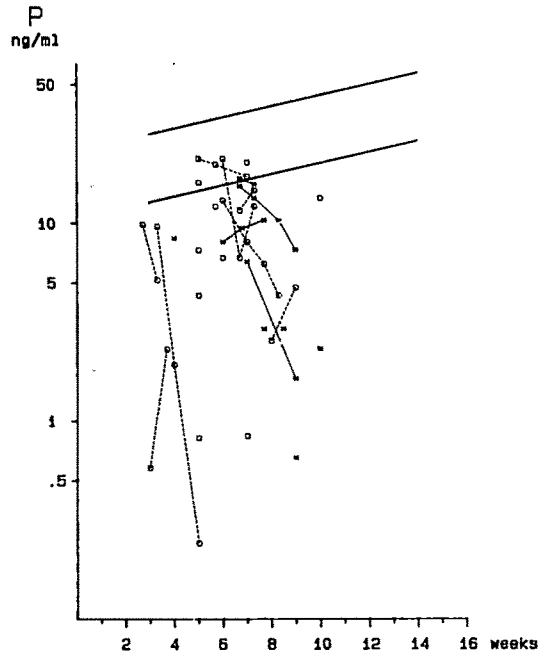


Fig. 2. Serum progesterone *(P)* concentration in patients with ectopic pregnancy. For further details refer to legend of Fig. 1.

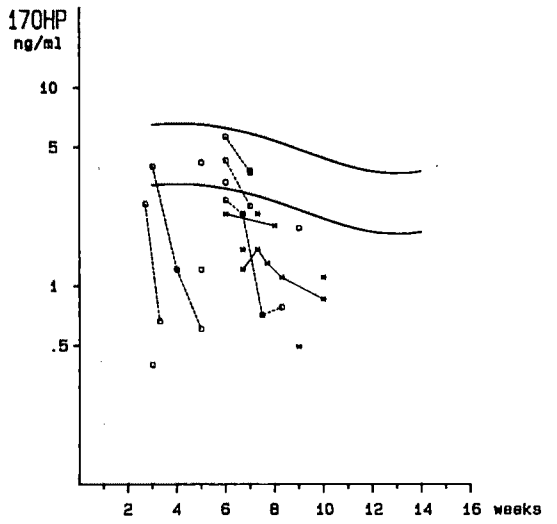


Fig. 3. Serum 17-hydroxyprogesterone *(17OHP)* concentration in patients with ectopic pregnancy. For further details refer to legend of Fig. 1.

that assumption, ectopic and normal pregnancies were paired for human chorionic gonadotropin, and progesterone levels were compared. As shown in Table II, in 20 of 21 cases a normal pregnancy could be found with a human chorionic gonadotropin value that matched within 4%; in the last case (121 versus 133 ng/ml) the difference reached 10%. The mean values of human chorionic gonadotropin were 580 and 583 ng/ml, respectively, for ectopic and normal pregnancies, and the paired t test did not show any significant difference between these two groups of patients. On the other hand, the progesterone concentration reached a mean value of only 7.8 ng/ml in ectopic pregnancy versus 23.6 ng/ml for the control group. As shown by the paired t test that difference was highly significant ($t = 5.622$, $p < 0.001$ for 20 df).

The data for the other steroids are also presented in Table II. For estradiol the mean values were 0.178 and 0.488 ng/ml ($t = 5.738$, $p < 0.001$ for 20 df), respectively, and for 17-hydroxyprogesterone they were 1.70 and 4.97 ng/ml ($t = 6.509$, $p < 0.001$ for 11 df).

The discrepancy between human chorionic gonadotropin and ovarian steroids was not specific for ectopic pregnancy; indeed similar results were obtained in a group of 20 patients with spontaneous abortion.

Comment

The results of serum human chorionic gonadotropin assays are in agreement with those of recent investigations.[1-6] Serial human chorionic gonadotropin determinations show a sensitivity of nearly 100%. Therefore the diagnosis of ectopic pregnancy may be ruled out when human chorionic gonadotropin is not detectable. Sandvei et al.[2] have reported, in a study of 100 cases,

Table II. Human chorionic gonadotropin, estradiol, progesterone, and 17-hydroxyprogesterone in the serum of patients with ectopic pregnancy of 7 or 8 weeks and in the sera of normal pregnancies paired for human chorionic gonadotropin concentration

Ectopic pregnancies				*Normal pregnancies*			
Human chorionic gonadotropin (ng/ml)	*Estradiol (ng/ml)*	*Progesterone (ng/ml)*	*17-Hydroxy-progesterone (ng/ml)*	*Human chorionic gonadotropin (ng/ml)*	*Estradiol (ng/ml)*	*Progesterone (ng/ml)*	*17-Hydroxy-progesterone (ng/ml)*
4.2	0.11	2.5	–	4.4	0.26	23.0	5.1
52	0.04	0.8	–	52.5	0.42	37.5	6.4
71	0.16	2.9	–	68	0.41	24.5	5.7
115	0.11	6.2	1.5	111	0.25	31.7	3.6
121	0.19	6.6	–	133	0.24	8.3	4.0
225	0.20	12.1	2.4	223	0.59	37.7	10.6
262	0.14	9.4	–	262	0.36	38.0	5.6
653	0.12	15.2	1.2	638	0.58	40.0	6.3
698	0.29	16.8	3.7	702	0.37	19.0	5.3
880	0.46	2.9	–	880	0.32	15.9	2.1
900	0.37	15.6	2.3	910	0.63	25.2	5.4
1,010	0.60	4.3	0.8	1,000	0.80	30.5	6.1
1,078	0.35	16.7	–	1,072	0.45	18.7	4.6
1,277	0.13	13.2	1.5	1,300	0.85	39.7	–
1,410	0.11	6.1	0.7	1,447	0.60	17.6	5.1
1,607	0.32	10.2	2.0	1,604	0.49	14.5	2.3
1,629	0.21	7.9	2.3	1,633	0.47	21.6	7.2
1,664	0.14	10.2	1.3	1,691	0.39	24.0	4.1
1,736	0.54	19.8	3.8	1,757	0.78	25.0	7.0
1,985	0.13	–	1.1	1,963	0.90	15.6	4.9
3,018	0.40	11.5	–	3,056	0.46	31.5	–
15,030	–	15.0	–	15,000	0.88	17.4	4.2

Table III. Predictive value of serum progesterone determination in early pregnancy

Pregnancy outcome	*Serum progesterone*					
	<15 ng/ml		*>15 ng/ml*		*Total*	
Normal		64		514		578
Abnormal						
Ectopic	9		2			
Abortion	41	54	21	30		84
Blighted egg	3		7			
Hydatidiform mole	1		–			
Total		118		544		662

on three patients with undetectable serum human chorionic gonadotropin levels. These were "devitalized" ectopic pregnancies. In the present investigation, only one patient had a human chorionic gonadotropin level that decreased from 10 ng/ml to undetectable; the diagnosis of tubal pregnancy was made fortuitously when laparoscopy and a microsurgical procedure were performed to manage a problem of infertility.

The "vitality" of ectopic pregnancy that eventually ruptures is probably better than that of those that do not rupture. This could explain both the higher hormone secretion and the trophoblastic proliferation leading to the tubal rupture.

As could be expected, luteal insufficiency is also associated with ectopic pregnancy. Most serum estradiol, progesterone, and 17-hydroxyprogesterone levels are lower than the mean normal values, with more than 80% of the data below the tenth percentile (Figs. 1, 2, and 3). Despite the presence of detectable amounts of human chorionic gonadotropin, which is presumed to stimulate the corpus luteum, the concentrations of these steroids were even lower than those in a normal luteal phase. The luteal insufficiency observed in ectopic pregnancy cannot be explained by the sole decrease of human chorionic gonadotropin. Indeed Fig. 4, which is derived from the data presented in Table II, shows that the concentration of ovarian steroids in ectopic pregnancy reaches only one third of

that observed in normal intrauterine pregnancy although both groups have been paired for identical human chorionic gonadotropin levels.

Such low values probably reflect the fact that, even in early pregnancy, a significant part of the so-called "ovarian steroids" is no longer secreted by the corpus luteum but by the trophoblastic tissue,[9] the vitality of which is critically reduced in ectopic pregnancy. However, other mechanisms may be involved in the reduction of steroid concentrations; for instance, one may suggest that insufficient stimulation of corpus luteum by human chorionic gonadotropin since the early stages of ectopic pregnancy induces its precocious senescence.

In cases of ectopic pregnancy the subsequent fertility of the patient is closely related to the time of the diagnosis. Therefore any investigation that could help an early diagnosis should be taken into consideration, particularly in patients at risk. To that regard the systematic quantitative determination of serum human chorionic gonadotropin levels is certainly a good precaution. The effectiveness of the human chorionic gonadotropin assay could be improved by serial determination as suggested by Kadar et al.[6] Indeed the interpretation of a single result may be difficult, especially in the case of an unreliable date of last menstrual period. Another way to improve an early diagnosis of ectopic pregnancy could be the simultaneous determination of human chorionic gonadotropin and progesterone. In normal pregnancy the progesterone level remains quite stable during the first weeks[10]; therefore a single result can be interpreted, even in the absence of an accurate gestational age, while this is not true for human chorionic gonadotropin. In the present investigation 84% of the first progesterone determinations fell below the tenth percentile (15 ng/ml). To assess the predictive value of the progesterone assay for an early diagnosis of ectopic pregnancy and threatened abortion, a prospective study was performed during a period of 15 months. During that period human chorionic gonadotropin and ovarian steroids were measured systematically at the first visit in 662 patients with a gestational age of ≤8 weeks. The correlation between progesterone concentration and pregnancy outcome is presented in Table III. As shown in that table a cutoff at 15 ng/ml gives a specificity of 88.9% (514 of 578) and a sensitivity of 64.7% (54 of 84). The predictive value for a progesterone level >15 ng/ml (percent of normal outcomes when progesterone concentration is normal) reaches 94.4% (514 of 544); that for a progesterone level <15 ng/ml (percent of abnormal pregnancies when progesterone concentration is subnormal) stands at 45.7% (54 of 118). The results of that prospective study confirm that the determination of the serum progesterone concentration in early pregnancy is a valuable tool for the screening of ectopic pregnancies and spontaneous abortions. However, it should be emphasized that human chorionic gonadotropin assays cannot be replaced by progesterone assays; the former remains essential to assume the presence of trophoblastic tissue somewhere in the patient, and the latter only provides some quantitative information on the "vitality" of the pregnancy. It is also clear that as soon as an intrauterine pregnancy can be visualized by ultrasonography such a policy is no longer necessary as regards the diagnosis of an ectopic pregnancy.[5, 11] However, hormonal investigations are of value in cases of threatened abortion or other abnormal bleeding.[12, 13]

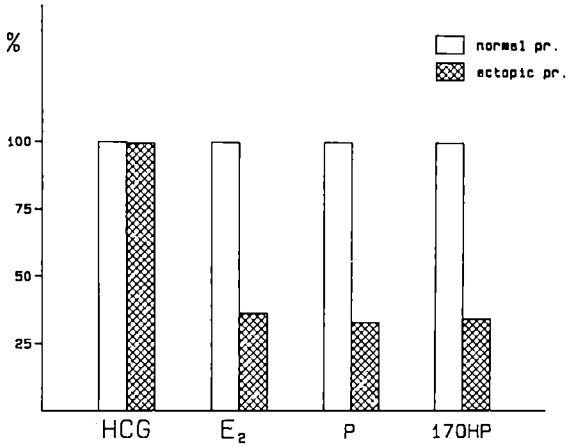


Fig. 4. Mean concentration of human chorionic gonadotropin *(HCG)*, estradiol *(E_2)*, progesterone *(P)*, and 17-hydroxyprogesterone *(17OHP)* in the serum of patients with normal *(open column)* and ectopic *(hatched column)* pregnancy. Samples have been paired for human chorionic gonadotropin concentration. Results are presented as percent of the geometric mean from the control group.

REFERENCES

1. Schwartz RO, Dipietro DL. HCG as a diagnosis aid for suspected ectopic pregnancy. Obstet Gynecol 1980; 56:197.
2. Sandvei R, Stoa KF, Ulstein M. Radioimmunoassay of human chorionic gonadotropin subunit as an early diagnosis test in ectopic pregnancy. Acta Obstet Gynecol Scand 1981;60:389.
3. Jouppila P, Tapanainen J, Huhtaniem I. Plasma HCG and ultrasound in suspected ectopic pregnancy. Eur J Obstet Gynecol Reprod Biol 1980;10:3.
4. Braunstein GD, Asch RH. Predictive value analysis of measurement of human chorionic gonadotropin, pregnancy specific β-glycoprotein, placental lactogen and cystine aminopeptidase for the diagnosis of ectopic pregnancy. Fertil Steril 1983;39:62.
5. Kadar N, Devore G, Romero R. Discriminatory HCG zone: its use on the sonographic evaluation for ectopic pregnancy. Obstet Gynecol 1980;58:156.
6. Kadar N, Calowell BV, Romero R. Method of screening for ectopic pregnancy and its indications. Obstet Gynecol 1981;58:162.

7. Milwidsky A, Adoni A, Segal S, et al. Chorionic gonadotropin and progesterone levels in ectopic pregnancy. Obstet Gynecol 1977;50:145.
8. Yates F. Contingency tables involving small numbers and the χ^2 test. J R Stat Soc 1934;1(suppl):217.
9. Csapo AI, Pulkkinen MO, Wiest WG. Effect of luteectomy and progesterone replacement in early pregnant patients. AM J OBSTET GYNECOL 1973;115:759.
10. Corker CS, Michie E, Hobson B, Parboosingh J. Hormonal patterns in conceptual cycles and early pregnancy. Br J Obstet Gynaecol 1976;83:489.
11. Gleicher N, Giglia RV, Depre G, et al. Direct diagnosis of unruptured ectopic pregnancy by real time ultrasonography. Obstet Gynecol 1985;61:425.
12. Aspillaga MO, Whittaker PG, Grey CE, Lind T. Endocrinologic events in early pregnancy failure. AM J OBSTET GYNECOL 1983;147:903.
13. Gerhard I, Runnebaum B. Predictive value of hormone determination in the first half of pregnancy. Eur J Obstet Gynecol Reprod Biol 1984;17:1.

Pregnancy-induced changes in the fibrinolytic balance: Evidence for defective release of tissue plasminogen activator and increased levels of the fast-acting tissue plasminogen activator inhibitor

Margaret Gore, M.D., Susan Eldon, A.B., Kenneth F. Trofatter, M.D., Ph.D., Seng-Jaw Soong, Ph.D., and Salvatore V. Pizzo, M.D., Ph.D.

Durham, North Carolina, and Birmingham, Alabama

Pregnancy is accompanied by an increased risk of thromboembolic disease. One contributing factor to such disease in the nonpregnant patient is disordered fibrinolysis. It has been suggested that defective fibrinolysis may occur in pregnancy, but this defect has been poorly characterized. In the present stucy of 52 women with normal pregnancies and 56 nonpregnant control women, we found a marked change in levels of releasable tissue plasminogen activator in pregnant women beginning in the first trimester. Whereas nonpregnant women demonstrated releasable levels of tissue plasminogen activator of 0.74 ± 0.15 IU/ml of plasma, in the pregnant women the amount released was only 0.06 ± 0.02 IU/ml of plasma ($p < 0.01$). These levels were observed beginning in the first trimester. Levels of the recently described fast-acting tissue plasminogen activator inhibitor increased significantly throughout pregnancy. Values ranged from 8.40 ± 0.27 IU/ml of plasma in the first trimester to 9.92 ± 0.09 IU/ml of plasma in the third trimester ($p < 0.05$) compared with the level of 8.46 ± 0.19 IU/ml of plasma in nonpregnant subjects. These data suggest that alterations both in releasable tissue plasminogen activator and in the fast-acting tissue plasminogen activator inhibitor contribute to the physiologic hypercoagulable state of pregnancy. (AM J OBSTET GYNECOL 1987;156:674-80.)

Key words: Fibrinolysis, tissue plasminogen activator, tissue plasminogen activator inhibitor, pregnancy

Uncomplicated pregnancies are accompanied by hypercoagulability and an increased risk of thromboembolic disease.[1, 2] Estimates of the incidence of thromboembolic disease in pregnant women have varied between 0.3 and 14 per 1000 pregnancies.[3, 4] Thrombosis is rare in the first trimester, and most events are noted in the last trimester. Interestingly, some patients demonstrate a thrombotic event in the face of a hemorrhagic tendency. In one study 46% of the pregnant women who had pulmonary emboli also demonstrated a hemorrhagic diathesis.[3] Complications included pelvic and perineal hematomas and placenta previa. A number of coagulation alterations are observed during pregnancy, including altered platelet function and increased plasma levels of fibrinogen and factors VII,

From the Departments of Pathology, Biochemistry, Obstetrics, and Gynecology, Duke University Medical Center, and the Department of Biostatistics, Comprehensive Cancer Center, University of Alabama.
This study was supported by Research Grants HL-24066 and HL-31932.
Received for publication August 4, 1986; revised September 22, 1986; accepted October 17, 1986.
Reprint requests: Dr. S. V. Pizzo, Duke University Medical Center, Box 3712, Durham, NC 27710.

VIII, IX, X, and XII.[1,2] Moreover, increased fibrinogen catabolism has been documented by a rise in plasma fibrin monomer complexes and split products.[1,2] It has been suggested that this hypercoagulability is a normal consequence of the need to protect the placental implantation site.[2]

Whereas levels of coagulation factors increase in pregnancy, deranged fibrinolysis may also contribute to the hypercoagulable state.[1,2] The nature of this altered balance is uncertain because the studies were performed with integrative assays of fibrinolysis such as the euglobulin lysis time or fibrin plate assay. Considerable advances in fibrinolytic research have been made during the last few years, and greatly improved and more specific assays of fibrinolysis are available.[5-8] It is necessary, in addition to measuring baseline plasminogen activator levels, to stimulate release of tissue-type plasminogen activator from blood vessels. Little of this substance is found in the unperturbed circulation, yet the level of releasable tissue plasminogen activator bears the closest correlation to a thrombotic disorder.[5-8]

More recently, alterations in a newly discovered fast-acting tissue plasminogen activator inhibitor have been described in conjunction with thromboembolic diseases.[7,8] This inhibitor is the major plasma inhibitor of tissue plasminogen activator as well as urokinase. The remaining plasma proteinase inhibitors, such as α_2-macroglobulin, α_1-antiproteinase, antithrombin III, and α_2-plasmin inhibitor, inactivate tissue plasminogen activator at a relatively slow rate. Since tissue plasminogen activator clears from blood with a half-life of only 2 minutes, such inhibitors would have little opportunity to bind tissue plasminogen activator.[9] A recent study has suggested an increase in the plasma inhibitory capacity for tissue plasminogen activator, particularly in the third trimester of pregnancy.[10]

Finally, alterations of vitamin K–dependent protein C have been associated with altered fibrinolysis in several families with idiopathic thromboembolic disease and in experimental models.[11] Levels of this protein, however, remain normal in pregnancy, even in the third trimester.[12] In the present study we have focused on measurement of tissue plasminogen activator and its inhibitor. In all, plasma from 52 pregnant women, 56 nonpregnant women, and 62 men were compared by means of a new and more specific fibrin-stimulated tissue plasminogen activator assay.[6,7]

Material and methods

Subjects. The pregnant population consisted of 52 women with no evidence of preeclampsia, eclampsia, or overt thromboembolic disease. All subjects had reached at least 10 weeks' gestation before the study was begun. The population's mean age was 24.5 ± 5.3 years (SD). The only basis for excluding a subject was refusal to participate in the study or inability to obtain a blood sample. The control group consisted of 56 nonpregnant women with a mean age of 26.3 ± 4.8 years. None of these women had a history of thromboembolic disease, and none was receiving hormonal supplements. None had been pregnant within 1 year of the study. In addition, 62 normal men were included for comparison. Blood was taken from all subjects after informed consent was obtained, in accordance with a protocol approved by the Human Experimentation Committee at Duke University Medical Center. Previous studies demonstrated no differences in releasable tissue plasminogen activator[5-7] on the basis of blood group, race, age, or sex. In the present study data were not stratified for blood group or race. The age difference in study and control subjects was <2 years and was not further analyzed.

Fibrinolytic assays. Blood samples were obtained before and after 5 minutes of venous occlusion at a pressure midway between systolic and diastolic, as previously described.[5-7] The samples were quick-frozen in dry ice–ethanol and kept at −80° C until assayed. The plasma was subjected to euglobulin fractionation and then reacted with plasminogen and the plasmin substrate D-valine-leucine-lysine-*p*-nitroanilide hydrochloride, as previously reported.[5-7] The assay was modified by addition of 10 μl of fibrin monomer (3.75 mg/ml) to each 500 μl assay sample. Fibrin monomer was prepared as previously described.[13] Substrate hydrolysis was monitored by absorbance at $\lambda = 405$ nm. A tissue plasminogen activator standard curve was constructed with a National Institute of Biological Standards control tissue plasminogen activator preparation (No. 83/517), generously supplied by Dr. Henry Berger of the Wellcome Research Laboratories. Fast-acting tissue plasminogen activator inhibitor was assayed as previously reported[7,8] by titration of the plasma inhibitor with purified tissue plasminogen activator (to a final theoretical plasma concentration of 10 IU/ml). In this assay the concentration of one component (tissue plasminogen activator) of the bimolecular reaction must be held constant in order to estimate the second component (tissue plasminogen activator inhibitor).[7] This method may result in an underestimation of levels if the amount of tissue plasminogen activator added is insufficient to react with all of the inhibitor. As previously reported,[10] this assay generally has been performed by addition of tissue plasminogen activator at a concentration of 2 IU/ml of plasma. We recently modified the assay by the addition of 5 IU/ml of plasma.[7] Under these conditions higher apparent levels of the inhibitor were noted.[7] In the present study assays were initially performed by addition of tissue plasminogen activator at a concentration of 5 IU/ml of plasma. When these same plasma

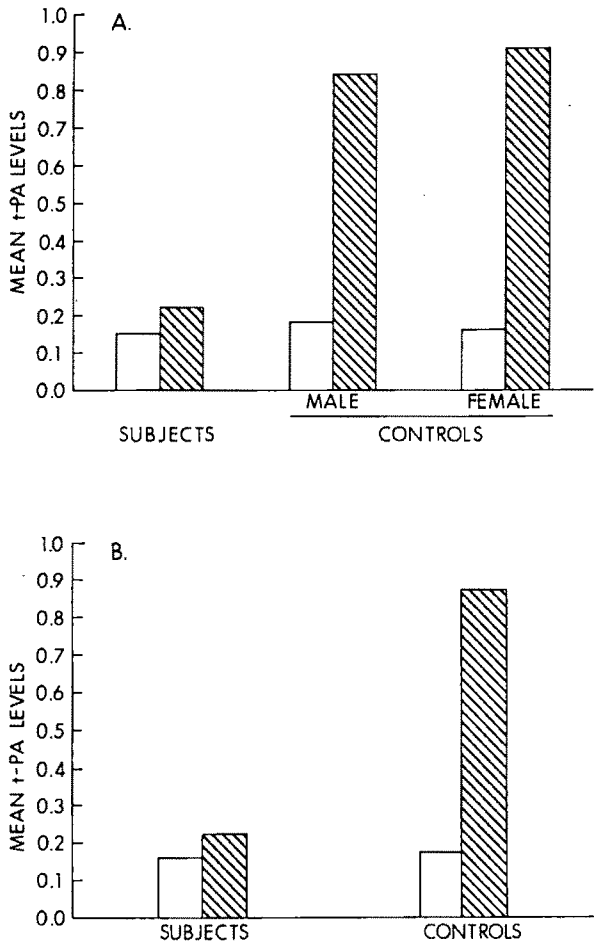


Fig. 1. Comparison of mean tissue plasminogen activator levels for pregnant women and control subjects. The *open bar* represents preocclusion levels of activator and the *hatched bar*, the postocclusion values. In *A* the controls are divided by sex, whereas in *B* a comparison is made to all 118 control subjects.

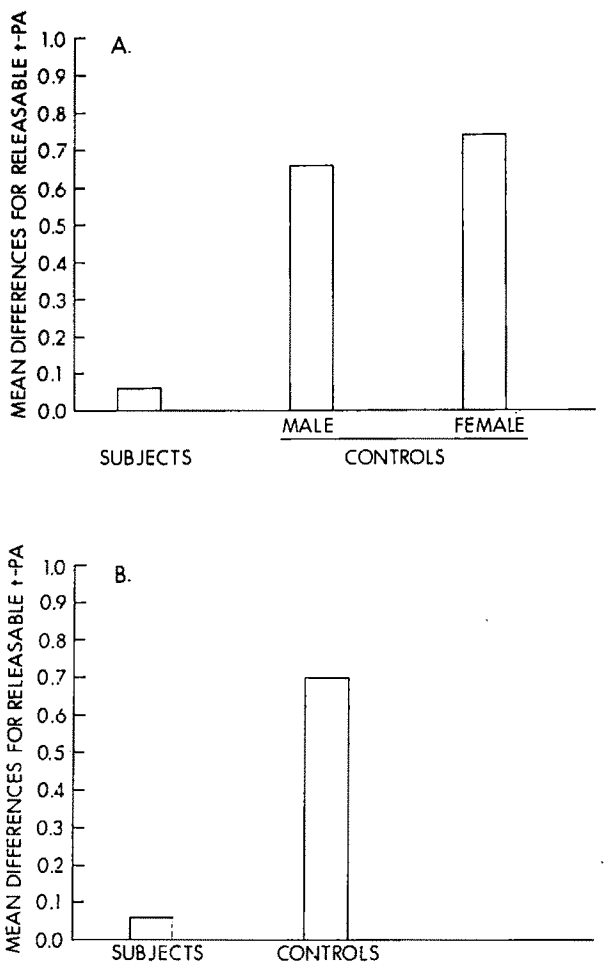


Fig. 2. Comparison of releasable tissue plasminogen activator levels for pregnant women and control subjects. *A* compares the pregnant subjects with men and nonpregnant women separately. In *B* comparison is made with all 118 control subjects.

samples were titrated with 10 IU/ml of plasma, the apparent inhibitor concentrations were often more than twofold higher but appeared to have reached a plateau. For this reason we have performed the assay by addition of tissue plasminogen activator at a concentration of 10 IU/ml of plasma to each sample of plasma. The same concentration of tissue plasminogen activator must be added to each sample or the assumptions for a bimolecular reaction mechanism will not be met.[7]

Statistical analysis. Student's *t* test, the paired *t* test, and Duncan's multiple range test were used in the statistical analyses, as indicated in the footnotes to the tables. For proper adjustment of significance level in the comparisons of all pairs of means involving more than two groups, Duncan's test was used. Mean values are reported along with the standard error of the mean, and all the statistical tests used were based on a two-tailed test.

Results

Tissue plasminogen activator. The mean values for plasma activator levels are presented in Fig. 1. Preocclusion levels of activator were essentially equivalent. By contrast, there was a significant difference ($p < 0.01$) between postocclusion levels of activator when pregnant women were compared with either nonpregnant women or men. Some of the activator present in plasma is urokinase-like, and subtraction of preocclusion from postocclusion plasma levels more clearly reflects release of vascular stores of tissue plasminogen activator.[5-7] Data on releasable tissue plasminogen activator are presented in Fig. 2.

These data were then analyzed for statistical significance (Table I). As anticipated, there was no significant difference between men and pregnant or nonpregnant women when preocclusion data were analyzed, with the mean values ranging from 0.16 ± 0.02 to 0.18 ± 0.02

Table I. Comparison of levels of plasminogen activators in pregnant women, nonpregnant women, and men

Measurement	*Pregnant women (n = 52) (IU/ml)*	*Control subjects*		*Comparisons**
		Men (n = 62) (IU/ml)	*Nonpregnant women (n = 56) (IU/ml)*	
Preocclusion activator	0.16 + 0.02†	0.18 ± 0.02	0.10 ± 0.02	No significant differences among 3 groups
Postocclusion activator	0.22 ± 0.03	0.84 ± 0.16	0.91 ± 0.16	No significant difference between control men and women; patients vs. control men: $p < 0.01$; patients vs. control women: $p < 0.01$
Releasable tissue plasminogen activator	0.06 ± 0.02	0.60 ± 0.14	0.74 + 0.15	No significant difference between control men and women; patients vs. control men: $p < 0.01$; patients vs. control women: $p < 0.01$
Fast-acting tissue plasminogen activator inhibitor	8.99 ± 0.17	8.63 ± 0.22	8.46 ± 0.19	No significant differences among 3 groups

*By Duncan's multiple range test.
†Mean ± SE.

Table II. Comparison of levels of plasminogen activators in pregnant women and all nonpregnant individuals

Measurement	*Pregnant women (n = 52) (IU/ml)*	*Men and nonpregnant women (n = 118) (IU/ml)*	*Comparisons**
Preocclusion activator	0.16 + 0.02†	0.17 ± 0.01	No significant difference
Postocclusion activator	0.22 ± 0.03	0.87 ± 0.11	$p < 0.001$
Releasable tissue plasminogen activator	0.06 ± 0.02	0.70 ± 0.10	$p < 0.001$
Fast-acting tissue plasminogen activator inhibitor	8.99 ± 0.17	8.55 ± 0.15	No significant difference

*By Student's *t* test.
†Mean ± SE.

IU/ml of plasma. When postocclusion data were compared values in the pregnant women (0.22 ± 0.03 IU/ml of plasma) were some fourfold lower than values for either men (0.84 ± 0.16 IU/ml of plasma) or nonpregnant women (0.91 ± 0.16 IU/ml of plasma). These differences were clearly reflected in the values of releasable tissue plasminogen activator that we calculated. Levels for pregnant women demonstrated a mean of only 0.06 ± 0.02 IU/ml of plasma compared with values of 0.60 ± 0.14 IU/ml of plasma in men ($p < 0.01$) or 0.74 ± 0.15 IU/ml of plasma in nonpregnant women ($p < 0.01$). These data reflect a tenfold to twelvefold decline in levels of releasable tissue plasminogen activator during pregnancy.

Since comparison of data for men and nonpregnant women demonstrated no significant differences for preocclusion or postocclusion levels of releasable tissue plasminogen activator, the two groups were combined and data compared for the entire group versus pregnant subjects (Table II). Since men tended to demonstrate slightly lower levels of releasable tissue plasminogen activator, this comparison would tend to weigh the interpretation against the difference hypothesis. However, the same results were obtained with an average releasable tissue plasminogen activator about elevenfold lower during pregnancy.

The data in Tables I and II may be interpreted as normal levels of plasminogen activators in a group of healthy subjects assayed by use of the most specific assays currently available and with fibrin monomer to stimulate tissue plasminogen activator. It is the largest group of normal individuals yet reported, with only a handful of subjects previously described by this type of assay.

The data from the pregnant subjects also were stratified by trimester of pregnancy (Table III). The acti-

Table III. Comparison of levels of plasminogen activators and fast-acting tissue plasminogen activator inhibitor by trimester of pregnancy

Measurement	*Trimester* 1 (n = 20)	2 (n = 17)	3 (n = 15)	*Comparisons**
Preocclusion activator	0.15 ± 0.03	0.14 ± 0.02	0.19 ± 0.04	No significant difference among 3 groups
Postocclusion activator	0.23 ± 0.08	0.18 ± 0.02	0.25 ± 0.05	No significant difference among 3 groups
Releasable tissue plasminogen activator	0.08 ± 0.06	0.04 ± 0.01	0.06 ± 0.02	No significant difference among 3 groups
Fast-acting tissue plasminogen activator inhibitor	8.40 ± 0.27	8.84 ± 0.35	9.92 ± 0.09	1 vs. 2: no significant difference; 1 vs. 3: $p < 0.05$; 2 vs. 3: $p < 0.05$

*By Duncan's multiple range test.

Table IV. Comparison of levels of plasminogen activators before and after delivery*

	Before delivery				*After delivery*			
Subject No.	*Before occlusion (IU/ml)*	*After occlusion (IU/ml)*	*Releasable tissue plasminogen activator (IU/ml)*	*Tissue plasminogen activator inhibitor (IU/ml)*	*Before occlusion (IU/ml)*	*After occlusion (IU/ml)*	*Releasable tissue plasminogen activator (IU/ml)*	*Tissue plasminogen activator inhibitor (IU/ml)*
1	0.12	0.13	0.01	9.94	0.22	0.63	0.41	9.73
2	0.06	0.09	0.03	8.25	0.22	0.23	0.01	6.12
3	0.07	0.29	0.22	8.68	1.46	1.73	0.27	8.74
4	0.16	0.24	0.08	9.89	0.49	1.77	1.28	9.25
5	0.22	0.32	0.10	9.16	0.23	0.56	0.23	8.36
6	0.19	0.39	0.20	8.99	0.32	2.04	1.72	9.66
7	0.20	0.31	0.11	9.83	0.17	0.50	0.37	7.89
Mean values ± SEM	0.15 ± 0.01	0.25 ± 0.02	0.11 ± 0.01	9.25 ± 0.11	0.44 ± 0.07	1.07 ± 0.11	0.61 ± 0.11	8.54 ± 0.21

*Comparisons of prepartum and postpartum values with respect to preocclusion, postocclusion, and releasable tissue plasminogen activator were all statistically significant ($p < 0.01$) by paired *t* test.

vator levels measured in the preocclusion and postocclusion samples as well as the calculated releasable tissue plasminogen activator demonstrated no significant differences. It is concluded that the changes in vascular stores of tissue plasminogen activator decline rapidly in pregnancy (at least from week 10) and then remain at low levels.

Plasminogen activators in the postpartum period. During the course of this study seven women reached term and were delivered of their neonates, offering an opportunity for assessment of tissue plasminogen activator in the immediate postpartum period. In these subjects blood was drawn 2 days after delivery. Although the numbers are small a dramatic change occurred after delivery (Table IV). Values for both preocclusion and postocclusion samples were increased after delivery, and releasable tissue plasminogen activator returned to near-normal levels by this time ($p < 0.01$).

Fast-acting tissue plasminogen activator inhibitor. Measurements of the fast-acting tissue plasminogen activator inhibitor present in plasma were obtained on the preocclusion plasma samples for the entire study group. These data indicate that men and nonpregnant women have essentially the same levels of circulating inhibitor (Table I). When the entire study group of pregnant women was considered, levels of inhibitor were not significantly altered, 8.99 ± 0.17 IU/ml of plasma versus 8.63 ± 0.22 IU/ml of plasma for men and 8.46 ± 0.19 IU/ml of plasma for nonpregnant women. However, when the data were stratified by trimester it became evident that levels of inhibitor rise with each trimester (Table III), with the largest difference occurring between the second and third trimesters. Comparison of either the first or second trimester versus the third trimester yielded a statistically significant difference ($p < 0.05$). Overall, inhibitor activity rose from 8.40 ± 0.27 IU/ml of plasma in the first trimester to 9.92 ± 0.09 IU/ml of plasma in the third trimester.

Levels of tissue plasminogen activator inhibitor were also measured in the seven available subjects after delivery (Table IV). Overall, for these women inhibitor levels fell from 9.25 ± 0.11 IU/ml of plasma in the third trimester of pregnancy to 8.54 ± 0.21 IU/ml of

plasma immediately after delivery. A trend is clearly evident in these data but does not reach the level of statistical significance ($p = 0.10$).

Comment

Previous studies have indicated alterations in both procoagulants and fibrinolytic activity during pregnancy.[1, 2] Most procoagulants increase in concentration during pregnancy,[1, 2] but the effect on fibrinolysis appears selective. Plasminogen levels increase significantly during pregnancy, peaking at week 28 of pregnancy.[1, 2] Levels remain elevated through about the first 8 weeks after delivery.[1, 2] By contrast, total fibrinolytic activity decreases as assessed by integrative assays of fibrinolysis, namely, the euglobulin lysis time[1, 2] or the fibrin plate method.[14] These data suggested that plasminogen activator levels might decrease in pregnancy; however, they are indirect measurements, and determination of activator is at best only semiquantitative. More recently, it has been suggested that tissue plasminogen activator inhibitor levels increase throughout pregnancy, particularly in the last trimester.[10] However, in this study only 2 IU tissue plasminogen activator per milliliter was added to titrate the inhibitor, and a number of normal subjects may exceed such levels, resulting in a spurious value for the inhibitor. In a subsequent study we found that this amount of tissue plasminogen activator was insufficient to titrate all the inhibitor.[7]

In the present study we have applied readily quantifiable assays of tissue plasminogen activator and its specific inhibitor to plasma from pregnant women and have compared the observed levels with those found in men and nonpregnant women. The inhibitor assay used a large excess of tissue plasminogen activator, 10 IU/ml, to titrate the inhibitor so that it is unlikely that inhibitor levels were underestimated. To date, this group ($n = 118$) is the largest cohort of normal subjects surveyed for tissue plasminogen activator and its newly described fast-acting inhibitor. These assays make it possible to dissect the nature of the fibrinolytic defect of pregnancy.

Our data indicate that a profound suppression in release of vascular tissue plasminogen activator occurs throughout the course of pregnancy. The limited data available after delivery suggest that there is a rapid return to normal levels of releasable activator. Basal levels of activator may actually be elevated immediately after delivery. These changes may be mediated by a number of pregnancy-associated changes in hormonal regulation. The elevation in plasma estrogen levels seen in pregnancy may suppress vascular stores of tissue plasminogen activator, although most data suggesting such a correlation relate to synthetic rather than natural estrogens.[15] The fall in estrogens around parturition may result in a rapid rebound in releasable tissue plasminogen activator. Rising levels of plasma oxytocin may also contribute to high basal levels of plasminogen activators because homologous peptides such as vasopressin and its analogues will trigger tissue plasminogen activator release.[16] Similarly, increases in plasma epinephrine concentration, likely to occur with the stress of delivery, also trigger release of vascular activator stores.[16]

The rise in plasma inhibitor levels may correlate with changes in the placenta itself. This tissue is a rich source of fibrinolytic inhibitors,[1, 2] and during the course of its maturation it may secrete increasing levels of inhibitors such as we observed in the third trimester. In this regard it is interesting to consider a possible role for defective production of placental inhibitor in the etiology of abruptio placentae or premature placental separation. In this setting there would be available circulating tissue plasminogen activator in the presence of high circulating plasminogen levels that might make the patient more prone to bleeding. Such bleeding in the retroplacental area may contribute to placental separation.

The rise in tissue plasminogen activator inhibitor levels observed in the present report is not as great as in the one previous study of this factor.[10] This difference may reflect variations in the assay conditions as this earlier study may not have used an adequate concentration of tissue plasminogen activator to quench all of the inhibitor present. It is apparent from the values reported[10] that a concentration of tissue plasminogen activator higher than the 2 IU/ml initially specified must have been added to some of the samples since values for inhibitor sometimes exceeded 2 IU/ml plasma. The kinetics of a bimolecular reaction require that one component be kept constant; otherwise the values may not be comparable. The present study suggests that the rise in tissue plasminogen activator inhibitor is not sufficient to account for the profound suppression in tissue plasminogen activator levels that was observed. This is particularly pertinent as this suppression was observed throughout pregnancy, whereas the inhibitor rise was seen only in the third trimester. Our data also suggest that tissue plasminogen activator inhibitor returns to nonpregnant levels relatively rapidly, although the data do not reach statistical significance at 2 days after delivery ($p = 0.10$). A major problem in this regard is the timing of the blood drawing during the third trimester. It is quite possible that levels would be higher than those we report if sampling were performed just before delivery. It would be very difficult to control for this variable since an extensive effort would be required to obtain multiple samples in the last trimester. It is apparent, however, that return to basal levels must occur over days rather than weeks.

Taken with previous studies, the present observations

suggest that a true hypercoagulable state exists in pregnancy. The immediate postpartum period presents the possibility of a rebound phenomenon since it is a time when high levels of plasminogen[4] may not be counterbalanced by low activator levels and high levels of inhibitor. It is conceivable that such alterations could at times contribute to postpartum bleeding in marked contrast to the prepartum thromboembolic tendency. These observations do not rule out the possibility that some women may remain in a hypercoagulable state for some weeks after delivery since a number of the procoagulant proteins such as fibrinogen and factor X remain elevated after delivery.[4] In many subjects as many as 8 weeks are required before the coagulation system returns to a normal balance. These hypotheses appear consistent with the paradox observed in some women in the early postpartum period who suffer a thromboembolic event while also manifesting a bleeding diathesis.

We thank Connie E. Hughes, R.N., for collecting blood samples from the subjects reported in this study.

REFERENCES

1. Kuhn W, Graeff H. Coagulation problems in pregnancy. Contrib Nephrol 1981;25:78.
2. Hellgren M, Blombäch M. Studies on blood coagulation and fibrinolysis in pregnancy, during delivery and in the puerperium. Gynecol Obstet Invest 1981;12:141.
3. Aaro LA, Juergens JL. Thrombophlebitis associated with pregnancy. AM J OBSTET GYNECOL 1971;109:1128.
4. Coon WW, Willis PW, Keller JB. Venous thromboembolism and other venous disease in the Tecumseh Community Health Study. Circulation 1973;48:839.
5. Gore M, Miller KE, Soong S-J, et al. Vascular plasminogen activator levels and thromboembolic disease in patients with gynecologic malignancies. AM J OBSTET GYNECOL 1984;149:830.
6. Campbell EE, Shifman MA, Lewis JG, et al. A new chromogenic assay for releasable plasminogen activator. Clin Chem 1982;28:1125.
7. Pizzo SV, Fuchs HE, Doman KA, et al. Release of vascular plasminogen activator and fast acting inhibitor of plasminogen activator in a patient with defective fibrinolysis. Arch Intern Med 1985;146:188.
8. Kruithof EKO, Tran-Thang C, Ransijn BF. Demonstration of a fast-acting inhibitor of plasminogen activators in human plasma. Blood 1984;64:907.
9. Fuchs HE, Berger H, Pizzo SV. Catabolism of human tissue plasminogen activator in mice. Blood 1985;65:539.
10. Wiman B, Csemiczky G, Marsh L, Robbe H. The fast inhibitor of tissue plasminogen activator in plasma during pregnancy. Thromb Haemost 1984;52:124.
11. Boekmans AW, Veltkamp JJ, Bertina RM. Congenital protein C deficiency and venous thromboembolism: a study of three Dutch families. N Engl J Med 1983;309:340.
12. Mannucci PM, Vigano S. Deficiencies of protein C, an inhibitor of blood coagulation. Lancet 1982;2:463.
13. Gonias SL, Pasqua JJ, Greenberg CS, Pizzo SL. Precipitation of fibrinogen, fibrinogen degradation products and fibrin monomer by histone H3. Thromb Res 1985;39:97.
14. Astedt B, Nilsson JM, Pandolfi M. Fibrinolytic activity in veins during pregnancy. Acta Obstet Gynecol Scand 1970;49:171.
15. Astedt B. Low fibrinolytic activity of veins during treatment with ethinyl estradiol. Acta Obstet Gynecol Scand 1971;50:279.
16. Collen D. On the regulation and control of fibrinolysis. Thromb Haemost 1980;43:77.

The effects of human chorionic gonadotropin on growth and steroidogenesis of the human fetal adrenal gland in vitro

Maysan Abu-Hakima, B.Sc., Charlotte L. Branchaud, Ph.D., Cynthia G. Goodyer, Ph.D., and Beverley E. Pearson Murphy, M.D., Ph.D.*

Montreal, Quebec, Canada

This study examined the effects of human chorionic gonadotropin, adrenocorticotropin, human luteinizing hormone, and mouse epidermal growth factor on growth (thymidine incorporation) and steroidogenesis (dehydroepiandrosterone sulfate production) of human fetal zone adrenal cells in monolayer culture. Two preparations of human chorionic gonadotropin extracted from pregnancy urine were used, one highly purified (National Institutes of Health, CR-121) and one less pure (Sigma). Thymidine incorporation was increased twofold to tenfold in cultures exposed to the Sigma human chorionic gonadotropin preparation or to mouse epidermal growth factor as compared to control. Pure National Institutes of Health human chorionic gonadotropin and luteinizing hormone had no effect on growth. When adrenocorticotropin was added alone or in combination with Sigma human chorionic gonadotropin or mouse epidermal growth factor, growth was decreased. Dehydroepiandrosterone sulfate production was stimulated by adrenocorticotropin but not by human luteinizing hormone, human chorionic gonadotropin, or mouse epidermal growth factor. These results suggest that human pregnancy urine contains a growth factor which remains to be identified but that pure human chorionic gonadotropin has no mitogenic or steroidogenic effects on the cultured fetal zone cells of the human fetal adrenal gland. (Am J Obstet Gynecol 1987;156:681-7.)

Key words: Fetal adrenal, growth, steroidogenesis, human chorionic gonadotropin, adrenocorticotropin

The unique pattern of growth and development as well as the steroidogenic capacity of the human fetal adrenal is well recognized,[1] but little is known about what regulates these activities in the definitive and fetal zones. Although a functional pituitary is important for maintenance of the fetal adrenal in the later stages of pregnancy, the normal appearance of the gland up to about 16 weeks in anencephalic fetuses[2] and the rapid involution of the fetal zone after birth[3] has suggested that extrapituitary factors may be involved in its development during early gestation.

Adrenocorticotropin (ACTH) is the principal pituitary factor controlling fetal adrenocortical function. There is also evidence that other peptides may be involved, including the placental hormone, human chorionic gonadotropin (hCG).[4-6] However, reports of the effects of hCG on growth and steroidogenesis in the fetal adrenal have been conflicting.[4-7]

In the present study, fetal zone cells of adrenal glands from midgestation human fetuses were maintained in monolayer culture. Growth and steroidogenic activity of these cells were examined during chronic exposure to a pure and an impure preparation of hCG, ACTH, human luteinizing hormone, or mouse epidermal growth factor. Growth was assessed by measuring the incorporation of tritiated thymidine into deoxyribonucleic acid, and steroidogenesis was characterized by dehydroepiandrosterone sulfate production.

Material and methods

Materials. ACTH$^{1-39}$ (pACTH) (Duracton, a long-acting preparation of porcine ACTH containing carboxymethylcellulose; 1 mU is approximately equivalent to 10 ng of synthetic human ACTH) was purchased from Nordic Biochemical Laboratories (Montreal, Quebec). Mouse epidermal growth factor was obtained from Collaborative Research (Lexington, Massachusetts) and purified hCG (CR-121) and human luteinizing hormone (LER-960) from the National Hormone and Pituitary Program of the National Institute of Arthritis,

From the Reproductive Physiology Unit, Montreal General Hospital, the Endocrine Research Laboratory, Montreal Children's Hospital, and the Centre for the Study of Reproduction, McGill University.

Supported by the Medical Research Council of Canada (C. L. B. and B. E. P. M.) and the Cancer Research Society (M. A. H.)

Presented in part at the Seventh International Congress of Endocrinology, Quebec City, Quebec, Canada, July 4, 1984 [Abstract No. 80].

Received for publication June 10, 1986; revised September 8, 1986; accepted October 8, 1986.

Reprint requests: Dr. B. E. Pearson Murphy, 1650 Cedar Ave., Montreal, Quebec, Canada H3G 1A4.

**Career Investigator, Medical Research Council of Canada.*

Diabetes, and Digestive and Kidney Diseases, National Institutes of Health (NIH), Bethesda, Maryland. The less pure hCG was obtained from Sigma Chemical Co. (St. Louis, Missouri); this is a crude preparation from human pregnancy urine involving minimal purification and displays a number of bands on electrophoresis (Sigma, personal communication). Ham's F-10 powdered culture medium and heat-inactivated fetal bovine serum were purchased from Grand Island Biological Company (Burlington, Ontario), amphotericin B (Fungizone) from Squibb Canada, Inc. (Montreal, Quebec), penicillin G (Crystapen) from Glaxco Laboratories (Montreal, Quebec), and gentamicin (Garamycin) from Schering (Pointe Claire, Quebec). Collagenase (type IA) and deoxyribonuclease were obtained from Sigma Chemical Co. (St. Louis, Missouri) and multiwell culture plates from Falcon Plastics (Los Angeles, California). Tritiated thymidine and dehydroepiandrosterone sulfate were purchased from New England Nuclear Canada Ltd. (Lachine, Quebec), and antiserum to dehydroepiandrosterone sulfate was obtained from Endocrine Sciences (Tarzana, California).

Preparation of monolayer cultures. Human fetal adrenal glands were obtained at the time of therapeutic abortion by dilatation and curettage (Department of Obstetrics and Gynecology, Hopital Notre-Dame, with approval from the local institutional ethics committees). Fetal age (gestational age less 2 weeks) was determined according to foot length.[8] The glands were placed in sterile vials containing cold Ham's F-10 medium and antibiotics and transported on ice to the laboratory.

After removal by dissection of any surrounding connective tissue, the capsule including the definitive zone was carefully removed from each gland. The remaining fetal zone was minced in dispersion medium consisting of 1 mg/ml collagenase and 0.1 mg/ml deoxyribonuclease in Ham's F-10 containing 10% fetal bovine serum and antibiotics. The cells were harvested every 20 minutes, spun down, and the pellet resuspended in culture medium (Ham's F-10 supplemented with 10% fetal bovine serum and antibiotics: 5 μg/ml of amphotericin B, 200 IU/ml of penicillin G, and 40 μg/ml of gentamicin). After two rinses the cells were distributed in multiwell (1.9 cm^2) plates in a volume of 0.5 ml (6 to 9 × 10^4 cells) per well. The plates were then incubated at 37° C in a humidified atmosphere with 5% carbon dioxide in air.

After 24 hours an additional 0.5 ml of culture medium was added. After 48 hours the plating medium was discarded and replaced with fresh medium. For the duration of each experiment the spent media were collected daily and replaced with 1 ml of fresh medium. Test substances were added directly to the culture wells in 20 μl of normal saline solution. Three experiments were performed, each with use of adrenals from two fetuses. The ages of the fetuses in experiment 1 were 14 and 15 weeks, in experiment 2 they were 13 and 15 weeks, and in experiment 3 they were 11 and 11.5 weeks. Experimental design was dictated by the amount of tissue available at any one time.

Tritiated thymidine incorporation. Growth of the fetal adrenal cells in culture was assessed by thymidine incorporation into deoxyribonucleic acid. During a period of active proliferation, before the cultures had reached confluence, each well received 1.0 ml of culture medium containing the appropriate test substance and 1 μCi of methyl–tritiated thymidine. The media were collected after 24 hours, and each well was rinsed three times with saline solution. To detach the cells, 0.5 ml of 0.6N sodium hydroxide was added to each well and the plates were incubated for a further 8 hours, after which the medium and detached cells were collected. To lyse the cells and precipitate the deoxyribonucleic acid, 0.5 ml of an ice-cold solution of 60% trichloroacetic acid was added to the cell suspension, giving a final concentration of 30% trichloroacetic acid. The trichloroacetic acid precipitates were stored at 4° C until assayed, when they were collected by rapid filtration with use of a Millipore 30254 sampling manifold (Millipore Corp., Bedford, Massachusetts). After the initial filtration, any unbound radioisotope was removed by washing the filters four times with ice cold 10% trichloroacetic acid. The filters were dried overnight at 37° C, placed in counting vials, and 4 ml of phosphotoluene scintillation fluid (4 gm of 2,5-diphenyloxazole (PPO) and 0.1 gm of 1,4-bis(5-phenyl-2-oxazolyl)-benzene (POPOP) per liter of distilled toluene) were added. The radioactivity was measured in an LKB Rackbeta II liquid scintillation counter. Final results were expressed as total counts per minute of tritiated thymidine taken up by the cells in each well.

Steroid production. Dehydroepiandrosterone sulfate and cortisol were measured by radioimmunoassay as described previously.[9] Because cortisol production by these cultures was very low or undetectable, the cortisol data are not presented.

Statistical analysis. Data from the thymidine incorporation and dehydroepiandrosterone sulfate studies were analyzed with analysis of variance followed by Duncan's multiple range test.

Results

Adrenal cell cultures. Freshly dispersed fetal zone cells from human fetal adrenal glands were successfully maintained in monolayer culture for up to 10 days. The cells attached within 48 hours of plating and retained their ability to secrete dehydroepiandrosterone sulfate. Mitotic activity was observed in the cultures throughout the incubation period. Epithelial cells were the predominant type found; very few fibroblasts were

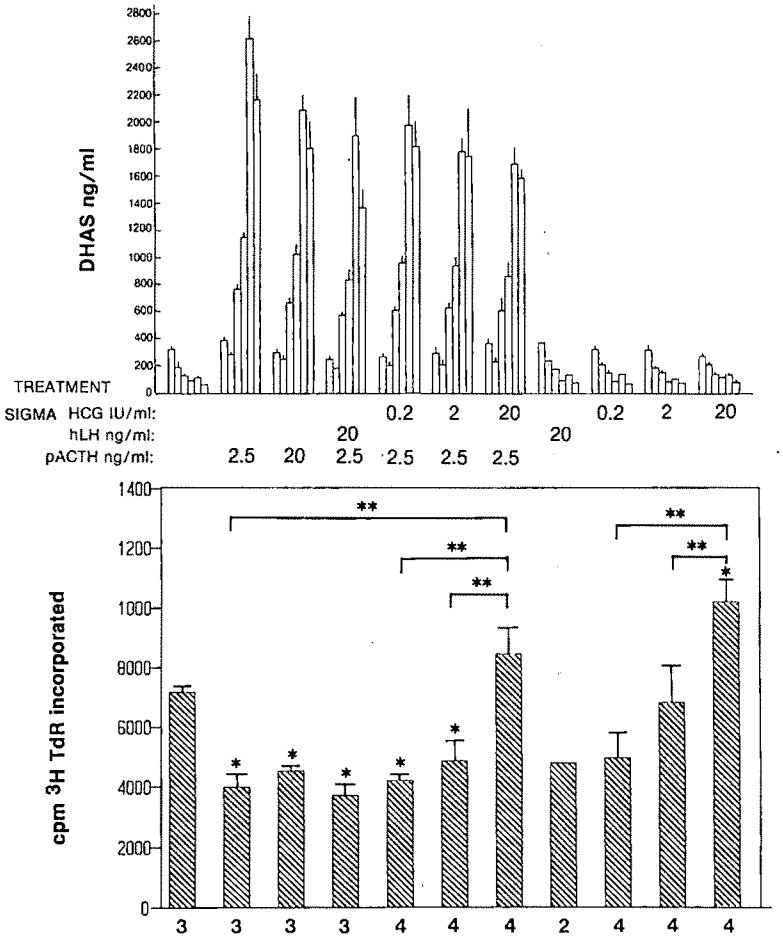


Fig. 1. *Upper panel:* The production of dehydroepiandrosterone sulfate on days 5 to 10 in culture by fetal zone cells from pooled adrenals of a 14- and a 15-week-old fetus. Each *bar* within a treatment group denotes the level of dehydroepiandrosterone sulfate per milliliter (mean ± SEM) produced during a 24-hour period. The first *bar*, representing day 5 of the experiment, shows the basal level. Test substances were added to the cultures daily from day 6 onward. *Lower panel:* The corresponding thymidine incorporation study for each treatment group (mean ± SEM), determined on day 11. *Numerical values* under the *bars* refer to the number of wells in each treatment group. Differences that were significantly different from control or between groups are indicated as *$p < 0.05$ and **$p < 0.01$.

seen. Large islands of what appeared to be adrenal medullary cells with extensive dendritelike connections were also present.

Tritiated thymidine incorporation. In experiment 1 (Fig. 1), both 2.5 and 20 ng/ml of pACTH inhibited ($p < 0.05$) tritiated thymidine uptake by the cultures. In the absence of pACTH, Sigma hCG significantly stimulated ($p < 0.05$) tritiated thymidine incorporation at 20 IU/ml but not at 0.2 or 2 IU/ml. In the presence of pACTH and the two lower doses of Sigma hCG, tritiated thymidine incorporation was similar to that observed in the presence of pACTH alone. The highest dose of Sigma hCG was able to block the inhibitory effects of pACTH. Human luteinizing hormone appeared to have an inhibitory effect at 20 ng/ml, but in the presence of 2.5 ng/ml of pACTH, thymidine uptake did not differ from that with pACTH alone.

In experiment 2 (Fig. 2), pure NIH hCG had no significant effect even at a dose of 1000 ng/ml. Mouse epidermal growth factor, however, at a dose of 10 ng/ml increased tritiated thymidine uptake more than threefold ($p < 0.01$). In the presence of pACTH at 2.5 ng/ml, mouse epidermal growth factor stimulation was reduced to a twofold increase but was still significant when compared to controls ($p < 0.05$).

In experiment 3 (Fig. 3), Sigma hCG (20 ng/ml) significantly increased tritiated thymidine incorporation over controls and was able to block the inhibitory effects of 2.5 ng/ml pACTH. Again pure NIH hCG had no significant effects at either dose level of 100 or 1000 ng/ml.

Dehydroepiandrosterone sulfate production. In experiment 1, production of dehydroepiandrosterone sulfate was determined from days 5 to 10 (Fig. 1). Con-

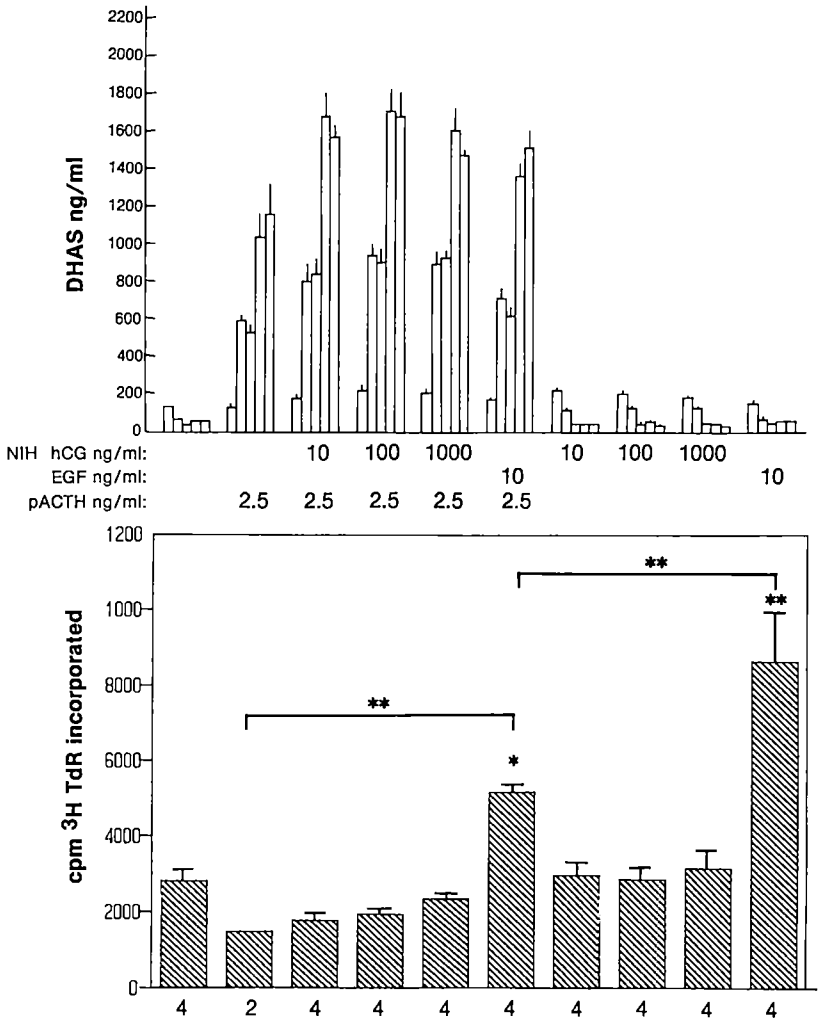


Fig. 2. Fetal adrenal cells were pooled from a 13- and a 15-week-old fetus. The experimental design was similar to that in Fig. 1, except that the *upper panel* shows the production of dehydroepiandrosterone sulfate on days 3 to 7 in culture. The first *bar*, representing day 3 of the experiment, shows the basal level of dehydroepiandrosterone sulfate production. Test substances were added daily from day 3 onward and thymidine incorporation *(lower panel)* was determined on day 8.

centrations of dehydroepiandrosterone sulfate on day 5 ranged from 250 to 399 ng/ml. On day 6 the levels were lower, with mean concentrations between 188 and 287 ng/ml. In the absence of any test substances, the levels of dehydroepiandrosterone sulfate in the control group continued to decline throughout the culture period, reaching a mean concentration of 66 ng/ml on day 10.

Beginning on day 6, various test substances were added to the culture medium. There was a marked increase in dehydroepiandrosterone sulfate when pACTH was added: the levels of dehydroepiandrosterone sulfate doubled in the first 24 hours after the addition of both the low (2.5 ng/ml) and high (20 ng/ml) doses of pACTH and continued to increase to peak levels (2000 ng/ml) on day 9 ($p < 0.01$ on day 10). No effects of luteinizing hormone or Sigma hCG were seen.

In experiment 2 the fetal zone cells were cultured for 7 days. The levels of dehydroepiandrosterone sulfate measured from days 3 to 7 are shown in Fig. 2. On day 3 the levels of dehydroepiandrosterone sulfate ranged from 130 to 208 ng/ml. In the control group the levels decreased to 38 ng/ml by day 5 and remained at this level until the end of the culture period.

Various test substances were added from day 3. In the 24 hours following the addition of pACTH (2.5 ng/ml), the concentration of dehydroepiandrosterone sulfate tripled. The levels of dehydroepiandrosterone sulfate increased steadily throughout the incubation period, reaching a maximum concentration of 1200 ng/ml on day 7 ($p < 0.01$).

Production of dehydroepiandrosterone sulfate by the fetal zone cells exposed to pure NIH hCG plus pACTH was comparable to that by cells treated with

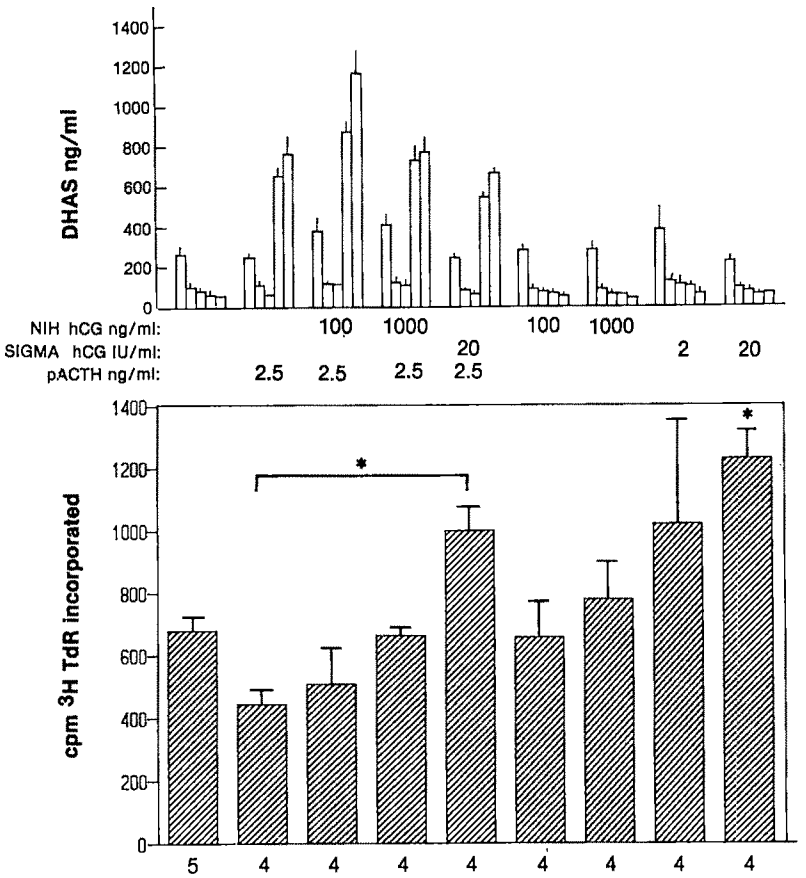


Fig. 3. Fetal adrenal cells were pooled from an 11- and an 11.5-week-old fetus. The experimental design was similar to that in Fig. 1, except that the *upper panel* shows the production of dehydroepiandrosterone sulfate on days 3 to 7. The first *bar*, representing day 3 of the experiment, shows the basal level of dehydroepiandrosterone sulfate production. Test substances were added daily from day 5 onward, and thymidine incorporation *(lower panel)* was determined on day 8.

pACTH alone. The pattern of dehydroepiandrosterone sulfate production by fetal zone cells exposed to mouse epidermal growth factor plus a low dose of ACTH (2.5 ng/ml) was also comparable to that by cells exposed to ACTH alone. When pure NIH hCG or mouse epidermal growth factor was added to fetal zone cells in the absence of ACTH, the levels of dehydroepiandrosterone sulfate from days 4 to 7 were similar to the pattern observed in the control group.

In experiment 3, fetal zone cells were maintained in culture for 7 days. As shown in Fig. 3, the dehydroepiandrosterone sulfate levels on day 3 ranged from 230 to 410 ng/ml in all the wells. In the control group, levels of dehydroepiandrosterone sulfate were reduced on day 4 and continued to decrease, reaching a plateau on day 5.

Test substances were added from day 5. Compared to the control group, there was an increase ($p < 0.01$) in dehydroepiandrosterone sulfate levels when the cells were exposed to pACTH (2.5 ng/ml) either alone or in combination with pure NIH hCG (0.1 or 1 μg/ml) or a high dose (20 IU/ml) of Sigma hCG. The pattern of dehydroepiandrosterone sulfate production in the presence of either NIH or Sigma hCG alone was similar to that observed in the control groups, in which basal levels of dehydroepiandrosterone sulfate were maintained.

Comment

The size of the fetal adrenal relative to body weight is enormously increased during gestation, reaching a peak around 15 weeks. at which time it equals the size of the fetal kidney; it remains large with another small increase in relative weight just before parturition.[1] The absolute weight increases steadily to a maximum just before delivery.[1] During the first month of postnatal life the fetal adrenal decreases in size by about half and thereafter grows proportionately less than other tissues.

Numerous studies in the child and adult have shown that ACTH administration in vivo results in increased adrenal weight as well as steroidogenic activity.[1] Similar

studies in the human fetus are obviously limited. Johannisson[4] has observed morphologic changes indicative of increased steroidogenic activity in both zones of the fetal adrenal of normal fetuses treated with ACTH in utero before termination of pregnancy at midgestation. Lanman[10] and Honnebier et al.[11] have reported an increase in both size and functional activity of the fetal zone in anencephalic newborn infants after ACTH treatment. However, in these anencephalic infants the fetal adrenal cortex appears to develop normally until about 16 weeks of gestation, even when serum ACTH levels are low or undetectable.[2] In addition, although there is a steady decrease in serum ACTH levels from midgestation to postnatal life during normal gestation,[12] it is only after birth that there is rapid involution of the fetal zone accompanied by the concomitant development of the adult zone. These observations have prompted the proposal that factors other than ACTH are involved in regulating growth of the fetal zone of the fetal adrenal. Indeed such factors have long been sought but remain to be identified.

Because it has not been feasible to study human fetal adrenal glands in vivo, extensive use has been made of in vitro methodologies[7, 12-14] to examine both steroidogenic activity and growth of the fetal adrenal cells. Using monolayers, Crickard et al.[15] have proposed that the growth-promoting effect of ACTH in vivo may be mediated by specific growth factors. They demonstrated that both mouse epidermal growth factor and bovine fibroblast growth factor were potent mitogens for human fetal adrenal cells. In the present study, mouse epidermal growth factor resulted in a threefold increase in the incorporation of thymidine into fetal zone cells from the midgestation gland, while it had no effect on dehydroepiandrosterone sulfate production. However, the observation that ACTH had a significant inhibitory effect on epidermal growth factor–stimulated proliferation of the cells suggests that the effects of ACTH and epidermal growth factor on growth may be antagonistic.

In view of the rapid postnatal involution of the fetal adrenal cortex, the fetoplacental unit is a likely source of adrenocorticotropic factors. It has been suggested that placental hCG may be one of these factors, although previous studies have produced conflicting data.[4-7] From the present findings it is clear that pure NIH hCG does not stimulate proliferation or steroidogenesis of the human fetal adrenal although this preparation demonstrated biologic activity in the rat ventral prostate weight gain assay. On the other hand, the less pure Sigma hCG preparation markedly stimulates thymidine incorporation with no effect on dehydroepiandrosterone sulfate production.

Since the Sigma hCG is prepared from pooled pregnancy urine, we postulate that the trophic effect of hCG we and others have observed may be mediated by a contaminating growth factor. Although the nature of this factor is not yet known, it is quite possibly placental in origin. This would be consistent with the concept of an involvement of extrapituitary factors in the regulation of adrenal development during gestation.

Since dehydroepiandrosterone sulfate is considered to be the principal steroid produced by the fetal zone in vitro,[6, 9, 13, 16] the detection of dehydroepiandrosterone sulfate in incubation media from the monolayer cultures was used as an index of steroidogenic activity. The only significant increase in dehydroepiandrosterone sulfate levels occurred in response to ACTH. Despite quantitative variability in this response, the pattern of endogenously released dehydroepiandrosterone sulfate was similar in all three experiments and is in agreement with the observations of others.[13, 14, 16] The decline in dehydroepiandrosterone sulfate levels observed with time in the absence of ACTH provides further support for the lack of steroidogenic factors in fetal bovine serum. The present results support the concept that ACTH is the principal factor regulating androgen production by the fetal adrenal cortex. In addition, it is apparent that separate mechanisms control growth and steroidogenesis in the midterm gland.

We gratefully acknowledge the cooperation of Dr. Yves Lefebvre (Department of Obstetrics and Gynecology, Hopital Notre Dame) in obtaining fetal tissues.

REFERENCES

1. Neville AM, O'Hare MJ. The Human Adrenal Cortex, Berlin: Springer-Verlag, 1982.
2. Gray ES, Abramovich DR, Morphologic features of the anencephalic adrenal gland in early pregnancy. AM J OBSTET GYNECOL 1980;137:491.
3. Tahka H. On the weight and structure of the adrenal glands and the factors affecting them in children of 0-2 years. Acta Paediatr 1951;40:1.
4. Johannisson E. The foetal adrenal cortex in the human. Its ultrastructure at different stages of development and in different functional states. Acta Endocrinol (Copenh) 1968;130:1.
5. Lehmann WD, Lauritzen C. hCG + ACTH stimulation of *in vitro* dehydroepiandrosterone production in human fetal adrenals from precursor cholesterol and Δ5-pregnenolone. J Perinat Med 1975;3:231.
6. Seron-Ferré M, Lawrence CC, Jaffe RB. Role of hCG in regulation of the fetal zone of the human fetal adrenal gland. J Clin Endocrinol Metab 1978;46:834.
7. Fujieda K, Faiman C, Reyes FI, Winter JSD. The control of steroidogenesis by human fetal adrenal cells in tissue culture. III. The effects of various hormonal peptides. J Clin Endocrinol Metab 1981;53:690.
8. Munsick RA. Human fetal extremity lengths in the interval from 9 to 21 menstrual weeks of pregnancy. AM J OBSTET GYNECOL 1984;149:883.
9. Branchaud CL, Goodyer CG, Shore P, Lipowski LS, Lefèbvre Y. Functional zonation of the midgestation human fetal adrenal cortex: fetal versus definitive zone use of

progesterone for cortisol synthesis. AM J OBSTET GYNECOL 1985;151:271.
10. Lanman JT. The adrenal gland in the human fetus: an interpretation of its physiology and unusual developmental pattern. Pediatrics 1961;27:140.
11. Honnebier WJ, Jobsis AC, Swaab DF. The effect of hypophysial hormones and human chorionic gonadotropin on the anencephalic fetal adrenal cortex and on parturition in the human. J Obstet Gynaecol Br Commonw 1974;81:423.
12. Goodyer CG, Branchaud CL, Lefebvre Y. Ontogeny of the human fetal pituitary-adrenal axis. In: Saez JM, Bertrand J, Ducharme JR, Collu R, eds. Ontogenesis of the endocrine system. Paris: Institut National de la Santé et de la Recherche Médicale 1982;109:163.
13. Goodyer CG, Hall CG, Branchaud C, Giroud CJP. Exploration of the human fetal pituitary-adrenal axis: stimulation of cortisol and dehydrepiandrosterone sulfate biosynthesis by homologous pituitary in organ culture. Steroids 1977;29:407.
14. Simonian MH, Gill GN. Regulation of the fetal human adrenal cortex; effects of adrenocorticotropin on growth and function of monolayer cultures of fetal and definitive zone cells. Endocrinology 1981;108:1769.
15. Crickard K, Ill CR, Jaffe RB. Control of proliferation of human fetal adrenal cells in vitro. J Clin Endocrinol Metab 1981;53:790.
16. Carr BR, Parker CR Jr, Milewich L, Porter JC, MacDonald PC, Simpson ER. Steroid secretion by ACTH-stimulated human fetal adrenal tissue during the first week in organ culture. Steroids 1980;36:563.

Reliable criteria for the prenatal sonographic diagnosis of alobar holoprosencephaly

Michael F. Greene, M.D., Beryl R. Benacerraf, M.D., and Fredric D. Frigoletto, Jr., M.D.
Boston, Massachusetts

A series of 10 consecutive cases of alobar holoprosencephaly is described. The disorder was diagnosed prenatally by ultrasound according to two criteria: (1) a large central fluid collection in the fetal head, with no visible midline structures but with the presence of a mantle around the fluid collection and fusion of the thalami and corpus striatum, and (2) sonographic abnormalities of the face, including hypotelorism, central clefts, facial asymmetry, and abnormal orbits. (AM J OBSTET GYNECOL 1987;156:687-9.)

Key words: Ultrasound, prenatal diagnosis, alobar holoprosencephaly

Alobar holoprosencephaly is a severe intracranial abnormality characterized by failure of proper cleavage of the prosencephalon, most often accompanied by incomplete midfacial development. The prenatal sonographic diagnosis of holoprosencephaly has been described[1, 2]; however it has been suggested that sufficient diagnostic accuracy has not yet been established so that appropriate obstetric management can be effected.[1]

We report here a series of 10 consecutive cases of alobar holoprosencephaly diagnosed prenatally with the use of two criteria. The intracranial criterion was a large central fluid collection in the fetal head, with no visible midline structures but with the presence of a mantle around the fluid collection and fusion of the thalami and corpus striatum (Fig. 1). The second criterion was sonographic abnormalities of the face, which included hypotelorism, central clefts, facial asymmetry, and abnormal orbits (Fig. 2). Ten consecutive cases, ranging from 16 to 38 weeks' gestation, satisfied both the intracranial and facial criteria for the diagnosis of alobar holoprosencephaly. The final diagnosis in all 10 cases was confirmed after either elective abortion or delivery (Table I). Karyotyping was done in nine of 10 cases, revealing four instances of trisomy 13 and one of triploidy. Three fetuses were aborted, one died in utero, and the other six died within 2 months of life. In the two additional cases where the presumptive diagnosis of holoprosencephaly was suggested on the basis of intracranial criteria but where no facial abnormality could be seen sonographically, after birth the infants were found to have severe hydrocephalus.

Alobar holoprosencephaly is a severe intracranial abnormality with a very poor prognosis. Affected infants usually die shortly after birth, although some can survive for 6 months and are profoundly retarded or have amentia.[3]

It is therefore crucial for the parents that this diag-

From the Department of Obstetrics and Gynecology, Brigham and Womens Hospital, Harvard Medical School.
Received for publication July 11, 1986; revised August 29, 1986; accepted September 9, 1986.
Reprint requests: Beryl R. Benacerraf, M.D., Diagnostic Ultrasound Associates, 333 Longwood Ave., Boston, MA 02115.

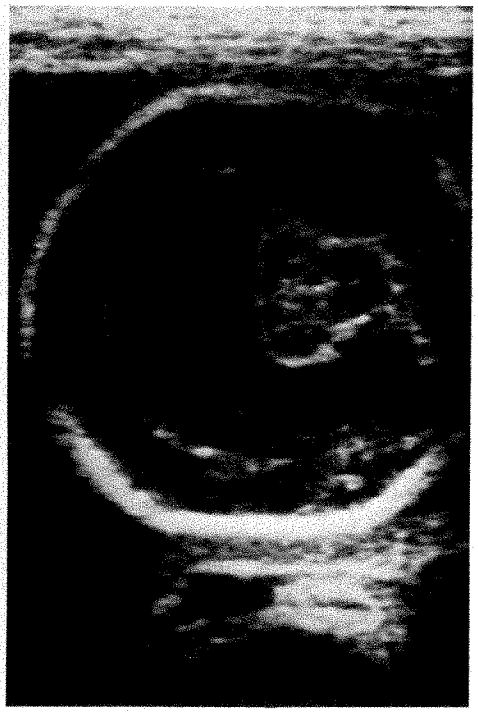


Fig. 1. Coronal view of the fetal head at 26 to 28 weeks' gestation showing a large central ventricle with a mantle of brain around it and abnormally fused thalami in the center (Case No. 1).



Fig. 2. Oblique longitudinal view of the fetal face showing a large central cleft (Case No. 10).

Table I. Findings in 10 cases

Case No.	*Gestational age at diagnosis (wk)*	*Ultrasound findings*	*Karyotype*	*Outcome*
1	26-28	Facial cleft and large central ventricle	Normal	Term delivery, neonatal death, findings confirmed at autopsy
2	16	Large central ventricle, cyclopia	Trisomy 13	Elective abortion, findings confirmed at autopsy
3	21	Twins, one normal, one with cyclopia, central ventricle, microcephaly	Normal	Normal twin survived, abnormal twin died at birth, findings confirmed at autopsy
4	26-27	Huge head, huge ventricle, fused thalami, abnormally small eyes, polyhydramnios	Normal	Term intrauterine death, findings confirmed at autopsy
5	32	Large central ventricle, cyclopia, oligohydramnios	Triploidy	Neonatal death, findings confirmed at autopsy
6	21	Large central ventricle, cyclopia	Trisomy 13	Elective abortion, findings confirmed at autopsy
7	30	Large central ventricle, asymmetry of orbits (small)	Normal	Findings confirmed at autopsy
8	19	Huge central ventricle, large midline cleft	Trisomy 13	Elective abortion, findings confirmed at autopsy
9	36	Microcephaly, central ventricle, abnormally small eyes and hypotelorism	Not done	Findings confirmed clinically at birth and radiographically, died at 2 mo, no autopsy done
10	38	Microcephaly, central ventricle, huge facial cleft and hypotelorism	Trisomy 13	Term delivery, neonatal death at 1 mo, findings confirmed at autopsy

nosis be made reliably in utero with a very high degree of accuracy so that alternatives of obstetric management can be offered. Our observations indicate that hydrocephalus and hydranencephaly can be severe enough to be confused with holoprosencephaly. We believe therefore that a careful examination of the face is necessary to provide the additional information required to diagnose reliably alobar holoprosencephaly in utero.

Occasionally a fetus with alobar holoprosencephaly can have normal or near normal facies.[1] It follows therefore that our criteria may not identify every fetus with holoprosencephaly. Also some fetuses can have severe midline facial abnormalities without the intracranial findings. If, however, a fetus fulfills both the intracranial and facial criteria described here for alobar holoprosencephaly, our data indicate that the diagnosis is sufficiently reliable that it can be used to offer the patient various obstetric options. Because the number of cases of holoprosencephaly presenting to a given laboratory is limited, confirmation of our findings in large numbers of patients will require further study.

REFERENCES

1. Chervenak FA, Isaacson G, Hobbins JC, et al. Diagnosis and management of fetal holoprosencephaly. Obstet Gynecol 1985;66:322.
2. Filly RA, Chinn DH, Callen P. Alobar holoprosencephaly: ultrasonographic prenatal diagnosis. Radiology 1984; 151:455.
3. Demeyer W, Zeman W. Alobar holoprosencephaly (arhinencephaly) with median cleft lip and palate: clinical, electroencephalographic and nosologic considerations. Confin Neurol 1963;23:1.

Fetal cardiac tumor: A prenatal echocardiographic marker for tuberous sclerosis

C. D. Gresser, R.N., R.D.M.S., J. Shime, M.D., H. Rakowski, M.D., J. F. Smallhorn, M.B., B.S., A. Hui, M.D., and J. J. Berg, M.B., M.Sc.

Toronto, Ontario, Canada

Auscultation of a fetal bradyarrhythmia led to the prenatal echocardiographic detection of an intracardiac tumor. After birth the diagnosis of tuberous sclerosis was established. Therapeutic implications for current and future pregnancies, when a fetal cardiac tumor is found, are discussed. (Am J Obstet Gynecol 1987;156:689-90.)

Key words: Fetal heart tumors, echocardiography, tuberous sclerosis

Cardiac neoplasms in the fetus may result in stillbirth or early neonatal death. As with infants and young children, these rare tumors are almost exclusively rhabdomyomas, although a case of right atrial hemangioma has been described at 28 weeks' gestation. In recent years, echocardiography has permitted identification of structural anomalies of the fetal heart including, to the best of our knowledge, five cases of cardiac tumor. We present an additional case in which the echocardiographic diagnosis was preceded by detection of a fetal bradyarrhythmia.

From the Divisions of Maternal-Fetal Medicine and Cardiology, Toronto General Hospital, and the Divisions of Cardiology and Genetics, Hospital for Sick Children, and the University of Toronto School of Medicine.
Supported by the Ontario Heart Foundation.
Received for publication June 3, 1986; revised August 8, 1986; accepted September 15, 1986.
Reprint requests: Dr. J. Shime, Room 223-6 Eaton Wing North, Toronto General Hospital, Toronto, Ontario, Canada M5G 1L7.

Case report

A healthy 31-year-old primigravid woman experienced an uneventful pregnancy until 32 weeks' gestation, when the fetal heart rate was noted to be slow and irregular. M-mode examination revealed an irregular bradycardia of approximately 100 bpm. Atrioventricular dissociation was sought but was not demonstrable. No structural anomaly was observed until the third examination at 34 weeks' gestation disclosed an echogenic 0.9 cm mass on the posterior aspect of the right atrium near the point of entry of the superior vena cava (Fig. 1). Another prominent echo was present at the base of a left ventricular papillary muscle. At 38 weeks, the bradycardia became more profound (baseline approximately 60 bpm), and ventricular function appeared to be reduced. Induction of labor was undertaken but progress was inadequate and cesarean delivery of a 3001 gm female infant was performed. Apgar scores were 8 and 9 at 1 and 5 minutes, respectively. Neonatal echo examination confirmed the right atrial

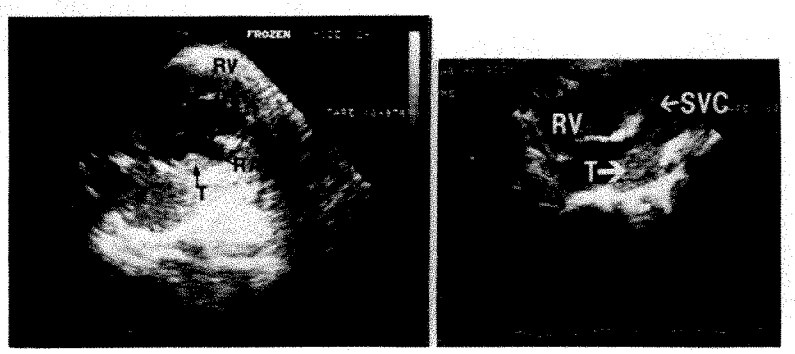


Fig. 1. Right atrial tumor noted in the fetal heart at 35 weeks' gestation. *Left:* Four-chamber view. *T,* Tumor mass; *RA,* right atrium; *RV,* right ventricle. *Right:* The tumor mass *(T)* is seen at the junction of the superior vena cava *(SVC)* and right atrium. *RV,* Right ventricle.

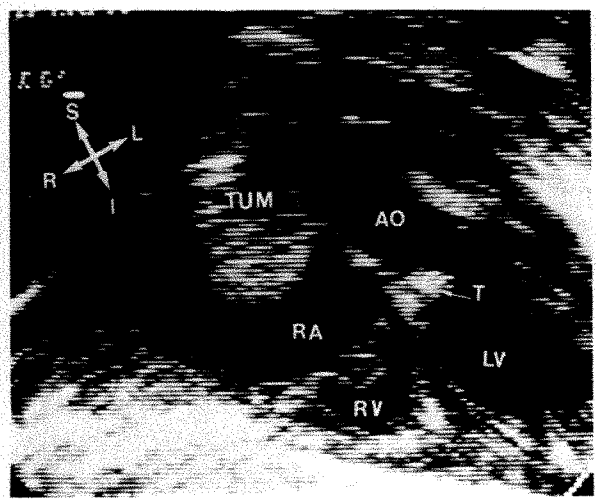


Fig. 2. Neonatal echo revealing right atrial tumor mass *(TUM)* and a smaller interventricular septal tumor *(T).*

tumor as well as smaller lesions in the left ventricle near the apex, at the base of the papillary muscle, and on the interventricular septum (Fig. 2). An electrocardiogram revealed a variable P-wave morphology consistent with a wandering atrial pacemaker. At 5 months of age, hypopigmented skin patches developed, leading to a diagnosis of tuberous sclerosis. The infant remained hemodynamically stable, and by 1 year of age, the right atrial mass appeared relatively smaller and the arrhythmia was less pronounced, with longer runs of sinus rhythm. Developmental, neurological, and renal assessments have remained normal. Family studies failed to reveal any evidence of tuberous sclerosis, thus raising the possibility that this case represents a new mutation.

Comment

Fetal cardiac tumors are rare. When symptomatic, they may present as (1) an arrhythmia secondary to a conduction defect or abnormal pacemaker function (as in our case), (2) nonimmunologic hydrops fetalis, or (3) death in utero from obstruction of blood flow by an intracavitary tumor. Tuberous sclerosis has been identified in up to one half of these patients and the diagnosis of fetal cardiac tumor should prompt a detailed ultrasound assessment to seek other abnormalities associated with this autosomal disorder, such as renal cysts or cerebral nodules. Pregnancy termination might be undertaken if multiple organ involvement is diagnosed early. In later pregnancy, if the fetus appears otherwise normal, hemodynamic impairment might dictate early delivery since neonatal resection of obstructive lesions has provided definitive therapy in several instances.[1] Whether tuberous sclerosis has occurred by new mutation or transmission from a carrier parent is crucial. In the latter instance, there is a 50% chance of recurrence in future offspring, with widely varying manifestations. For this reason, before embarking on future pregnancies, the parents should be offered prenatal genetic counseling, and during pregnancy, the previously described fetal assessment should be instituted.

This case and others illustrate that fetal cardiac tumors may present as arrhythmias and very often are markers for tuberous sclerosis.[2-4] Prenatal echocardiography may serve to define the arrhythmia, locate the tumor, and monitor hemodynamic changes.

REFERENCES

1. DeLoma JG, Villagra F, Perez De Leon JM, Casanova M, Collado R, Brito JM. Rhabdomyoma of the heart; surgical treatment. J Cardiovasc Surg 1982;23:149-54.
2. Birnbaum SE, McGahan JP, Janos GG, Meyers M. Fetal tachycardia and intramyocardial tumours. J Am Coll Cardiol 1985;6:1358-61.
3. Crawford E, Garrett C, Tynan M, Neville VG, Allan LD. Cardiac rhabdyomyomata as a marker for the antenatal detection of tuberous sclerosis. J Med Genet 1983;20:303-12.
4. DiLollo L, Castellani R, Maiorana A. Ultrasonic patterns of a cardiac rhabdomyoma detected in utero. J Perinat Med 1984;12:339-42.

FOR VULVOVAGINAL CANDIDIASIS

Think

vaginal cream 2.0%

Femstat®

(butoconazole nitrate)





FAST, SOOTHING RELIEF
Complete coverage–
and minimal vaginal leakage![1]

3-DAY THERAPY
As effective as miconazole cream–
in only 3 days' time!

3 DISPOSABLE APPLICATORS
Patient convenience and satisfaction–
for enhanced compliance!

*In non-pregnant patients, 3-day treatment is recommended. Treatment may be extended an additional 3 days, if necessary.

1. Droegemueller W, Adamson DG, Brown D et al: Three-day treatment with butoconazole nitrate for vulvovaginal candidiasis. *Obstet Gynecol* 64:530-534, 1984.

Please see brief summary of prescribing information on next page.

The Only 3-Day Cream*

Think

vaginal cream 2.0%

Femstat®

(butoconazole nitrate)

The Only 3-Day Cream

Brief Summary

INDICATIONS:
FEMSTAT® (butoconazole nitrate) vaginal cream 2.0% is indicated for local treatment of vulvovaginal mycotic infections caused by Candida species. Confirm the diagnosis by KOH smears and/or cultures. FEMSTAT can be used with oral contraceptives and antibiotics. It is effective in non-pregnant women and during the second and third trimesters of pregnancy.

CONTRAINDICATIONS:
FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

PRECAUTIONS:
General:
If clinical symptoms persist, repeat microbiological tests to rule out other pathogens and confirm the diagnosis. Discontinue drug if sensitization or irritation occurs.

Information for the Patient:
Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis:
Animal studies have not been done.

Mutagenesis:
Mutagenicity studies were negative.

Impairment of Fertility:
Animal studies showed no impairment of fertility.

Pregnancy Category C:
Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers:
Use with caution.

Pediatric Use:
Safety and efficacy have not been established.

ADVERSE REACTIONS:
Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

DOSAGE AND ADMINISTRATION:
Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

CAUTION:
Federal law prohibits dispensing without prescription.

December 1985

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

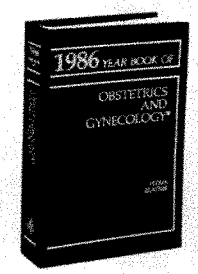
© 1986 Syntex Laboratories, Inc.

THE 1986 YEAR BOOK OF

OBSTETRICS AND GYNECOLOGY®

Edited by: Roy M. Pitkin, M.D., and Frank J. Zlatnik, M.D.

THE YEAR BOOK CONCEPT:



- Clinically oriented
- Condensations of the most vital journal articles worldwide chosen with your needs in mind—studies, discoveries, and developments that will affect your practice today. . .and tomorrow
- Pointed editorial comments on the articles—assessing their validity, relating them to other studies, and spotting new trends in the field
- A handsome, compact volume complete with illustrations, indexes, and references—a virtual storehouse of information enriching your professional library

1986 539 pp./229 illus. $42.95*

FREE ON 30-DAY APPROVAL!

Please rush me the following title for a free, trial examination:

____ **The 1986 Year Book of Obstetrics and Gynecology®**
(5-86) $42.95*

Dial toll-free 800/621-9262.
In Illinois, call collect (312) 726-9746.

NAME _____

ADDRESS _____

CITY _____

STATE _____ ZIP _____

PHONE _____

CIRCLE ONE: Visa/Master Card/AMX # _____

SIGNATURE _____

YEAR BOOK MEDICAL PUBLISHERS
35 EAST WACKER DRIVE
CHICAGO, ILLINOIS 60601

*This book is published annually. For the convenience of its customers, Year Book enters each purchaser as a subscriber to future volumes and sends annual announcements of each volume approximately two months before publication. The new volume will be shipped upon publication unless you complete and return the cancellation notice attached to the announcement and it is received by Year Book within the time indicated (approximately 20 days after your receipt of the announcement). You may cancel your subscription at any time. The new volume may be examined on approval for 30 days, may be returned for full credit, and if returned Year Book will then remove your name as a subscriber. Return postage is guaranteed by Year Book to the Postal Service.

Prices quoted are in U.S. dollars. Canadian orders will be billed in Canadian funds at the approximate current exchange rate. A small additional charge will be made for postage and handling. Illinois and Tennessee residents will be billed appropriate sales tax. All prices quoted subject to change. Title marked (†) is forthcoming; please do not send cash for this book.

ZZMISC

Maternal-Fetal Medicine

Hennepin County Medical Center and the University of Minnesota seek a faculty member to teach and conduct research in Maternal-Fetal Medicine. Candidates must have six years post M.D., board qualified or certified in Maternal-Fetal Medicine will be considered at the Assistant Professor level. Recruiting deadline is April 30, 1987.

Hennepin County Medical center and the University of Minnesota are equal opportunity employers and specifically invite and encourage applications from women and minorities.

Address inquiries to:

Bruce A. Work, Jr., M.D.
Professor and Head
Obstetrics and Gynecology
University of Minnesota
Box 395 UMHC
420 Delaware Street S.E.
Minneapolis, Minnesota 55455

UNIVERSITY OF GENEVA FACULTY OF MEDICINE

Applications are invited
for a tenured Faculty position at the
ASSOCIATE PROFESSOR (EXTRAORDINARIUS)
or
PROFESSOR (ORDINARIUS) level
in **Gynecological Endocrinology - Treatment of sterility**

The position is integrated in the Department of Obstetrics and Gynecology.

The appointee is expected to establish independent research in the area of reproductive endocrinology and treatment of sterility. The appointment starts on the 1st of October 1988.

Applicants should hold an M.D. degree with Board certificate or its equivalent. Application, including curriculum vitae, list of publications and a selection of 5-6 reprints (in 3 exemplars) should be sent to:

Secrétariat de la Faculté de Médecine
Hôpital cantonal universitaire
CH - 1211 GENEVA 4, Switzerland.

Closing date: May 1st, 1987.

FACULTY POSITION

The Department of Obstetrics and Gynecology at the University of Tennessee Medical Center at Knoxville, Tennessee is currently accepting applications for additional Maternal-Fetal Medicine Specialists.

These positions require Board certification or eligibility in Maternal Fetal Medicine. Responsibilities include: resident and medical student education, participation in Regionalization program and clinical or basic research.

Specialized areas of interest such as: critical care; ultrasonography and doppler studies; and prenatal diagnosis are encouraged. A private practice opportunity is available.

Academic rank is commensurate with qualifications and experience.

Interested persons should send curriculum vitae to:

Chairman, Department of
Obstetrics and Gynecology
University of Tennessee
Medical Center
1924 Alcoa Highway, U-27
Knoxville, TN 37920

For more information call
(615) 544-9306



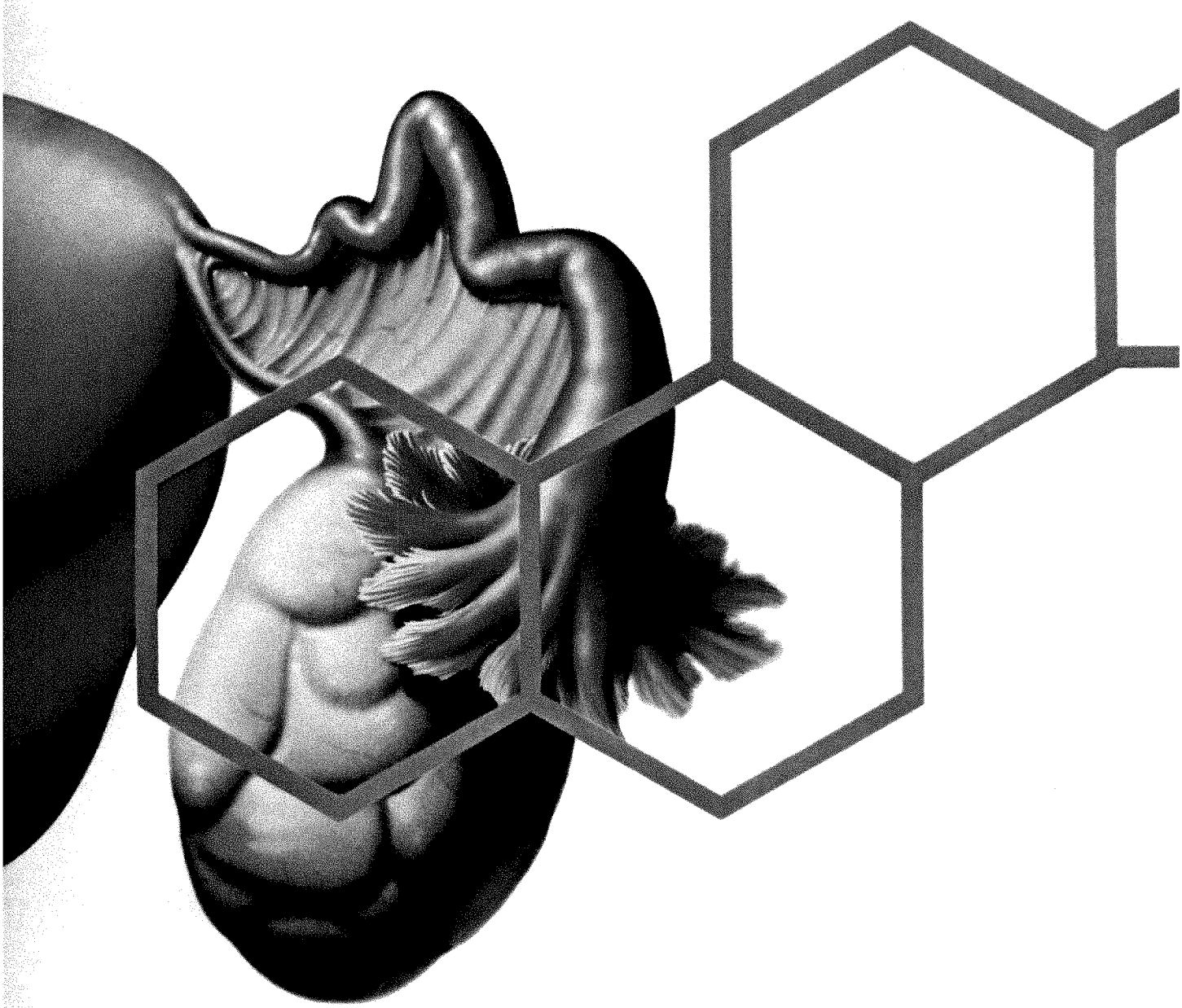
An Affirmative Action/Title IX/Section 504 Employer

OBSTETRICIAN/ GYNECOLOGIST
GREATER BOSTON OPPORTUNITY

Dynamic, highly motivated board eligible or board certified obstetrician/gynecologists sought to establish private practice in affiliation with a sophisticated 305 bed full service community teaching hospital 10 miles west of Boston. The ideal candidate will be one interested in a private practice supplemented by hospital based responsibilities. Generous financial assistance package is available to support the early years of practice. Position available immediately. Submit curriculum vitae and references to: Richard L. Brown, Executive Vice President and Chief Operating Officer, WalthamWeston Hospital & Medical Center, Hope Avenue, Waltham, MA 02254.

THE RIGHT CHEMISTRY

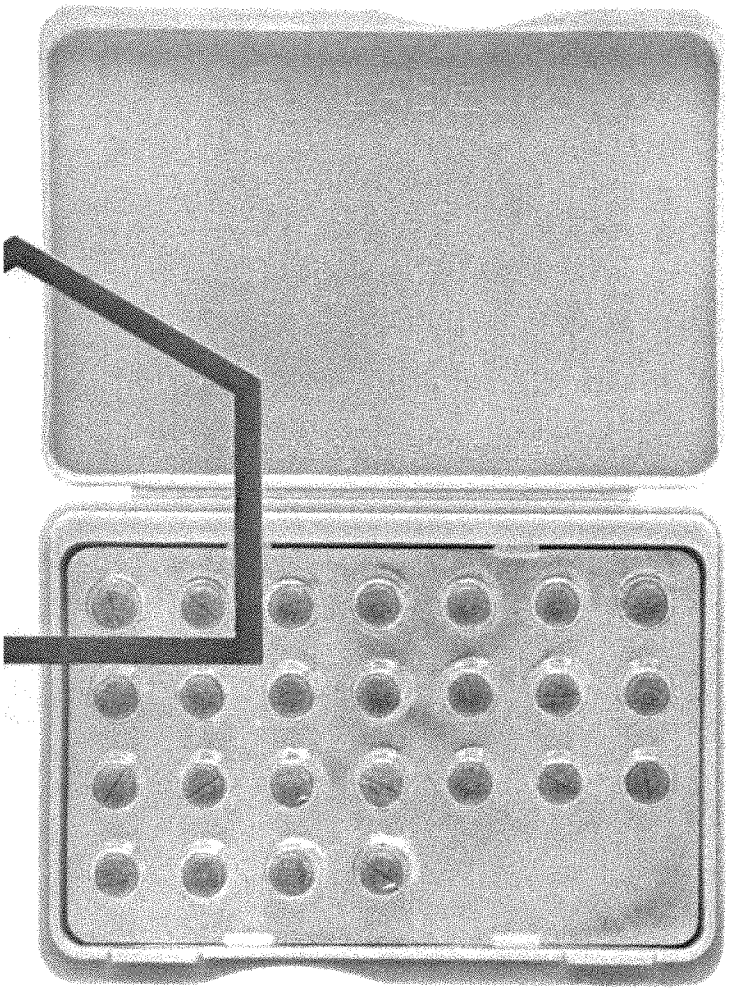
THE RIGHT REPLACEMENT FOR 17β-ESTRADIOL— 17β-ESTRADIOL



© 1986 Mead Johnson & Company • Evansville, Indiana 47721 USA MJL 5-4169

THE RIGHT REGIMEN

THE FIRST 25 DAYS OF THE MONTH— EVERY MONTH



ESTRACE is 17β-estradiol—just like the estrogen she made before menopause. Like the natural hormone, it follows a simple, well-known metabolic pathway, and it provides unsurpassed relief of postmenopausal vasomotor symptoms.

Micronized for good oral absorption, ESTRACE produces prompt, predictable serum levels of estradiol and estrone. Between cycles, ESTRACE is rapidly eliminated, providing estrogen replacement therapy that closely follows the premenopausal pattern . . . *for true cyclic therapy.*

Now it's in the ESTRACE Cycle Pak—an easy-to-carry compact, designed to deliver a starting dose of 2 mg in the preferred 25-day regimen. Cycle Paks delivering 1 mg daily are also available, if needed.

Mead Johnson LABORATORIES

For Brief Summary, please see following page.

ONLY NEW ESTRACE® (ESTRADIOL) TABLETS 1 mg and 2 mg CYCLE PAK HAS BOTH

ESTRACE® (estradiol)

TABLETS, 1 mg and 2 mg VAGINAL CREAM, 0.01%

Warning

1. **ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.** Three independent case control studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade. The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. **ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.** The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1000 exposures. Furthermore, a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb reduction defects. One case control study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed *in utero* to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. If ESTRACE® (estradiol) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation.

Indications: *Tablets:* Moderate to severe *vasomotor* symptoms associated with the menopause (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.); atrophic vaginitis; kraurosis vulvae; female hypogonadism; female castration; primary ovarian failure; breast cancer (for palliation only) in appropriately selected women and men with metastatic disease; prostatic carcinoma—palliative therapy of advanced disease. *Vaginal Cream:* Atrophic vaginitis and kraurosis vulvae. ESTRACE® HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

Contraindications: Known or suspected cancer of the breast, except in appropriately selected patients being treated for metastatic disease; known or suspected estrogen-dependent neoplasia; known or suspected pregnancy (See Boxed Warning); undiagnosed abnormal genital bleeding; active thrombophlebitis or thromboembolic disorders; a past history of thrombophlebitis, thrombosis or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

Warnings: Estrogens increase the risk of carcinoma of the endometrium (See Boxed Warning). Prescribe cautiously in women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms. A 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease has been reported in women receiving postmenopausal estrogens. There is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders or (except in treatment of malignancy) with a history of such disorders in association with estrogen use; they should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed. Large doses (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis; when estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptives should be considered a clear risk. Hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Increased blood pressure occurs with use of estrogens in the menopause, and blood pressure should be monitored, especially with high doses. Diabetic patients should be carefully observed for decreased glucose tolerance. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases; if this occurs, the drug should be stopped and appropriate measures taken to reduce the serum calcium level.

Precautions: *General*—A complete medical and family history should be taken prior to initiation of therapy. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination. Conditions influenced by fluid retention, such as epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc, may develop. Patients with a history of depression should be carefully observed. Pre-existing uterine leiomyomata may increase in size during estrogen use. Pathologists should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops, discontinue use while cause is investigated. Administer with caution in patients with impaired liver function, metabolic bone diseases associated with hypercalcemia, or renal insufficiency and in young patients with incomplete bone growth. The following endocrine and liver function tests may be affected by larger estrogen doses: increased sulfobromophthalein retention; increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability; increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay; free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered; impaired glucose tolerance; decreased pregnanediol excretion; reduced response to metyrapone test; reduced serum folate concentration; increased serum triglyceride and phospholipid concentration.
Information for the Patient—See text of Patient Package Insert.
Pregnancy Category X—See Contraindications and Boxed Warning.
Nursing Mothers—Administer to nursing mothers only when clearly necessary.
The 2-mg tablets contain FD&C Yellow No. 5 (tartrazine), which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals, frequently those who also have aspirin hypersensitivity.

Adverse Reactions: (See Warnings regarding induction of neoplasia, adverse effects on the fetus, increased incidence of gall bladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.) The following additional adverse reactions have been reported: *Genitourinary system*—Breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea; premenstrual-like syndrome; amenorrhea during and after treatment; increase in size of uterine fibromyomata; vaginal candidiasis; change in cervical eversion and in degree of cervical secretion; cystitis-like syndrome. *Breast*—Tenderness, enlargement, secretion. *Gastrointestinal*—Nausea, vomiting; abdominal cramps, bloating; cholestatic jaundice. *Skin*—Chloasma or melasma which may persist when drug is discontinued; erythema multiforme, erythema nodosum, hemorrhagic eruption, loss of scalp hair, hirsutism. *Eyes*—Steepening of corneal curvature; intolerance to contact lenses. *CNS*—Headache, migraine, dizziness; mental depression; chorea. *Miscellaneous*—Increase or decrease in weight, reduced carbohydrate tolerance, aggravation of porphyria, edema, changes in libido.

How Supplied: Tablets, containing 1 mg (lavender) and 2 mg (turquoise) micronized estradiol, in bottles of 100 and compacts of 25; Vaginal Cream, each gram containing 0.1 mg 17β-estradiol in a nonliquefying base containing purified water, propylene glycol, stearyl alcohol, white ceresin wax, glyceryl monostearate, hydroxypropyl methylcellulose, sodium lauryl sulfate, methylparaben, disodium edetate, and *t*-butylhydroquinone, in 1½-oz (42.5-g) tubes with calibrated plastic applicator for delivery of 1,2,3, or 4 g and in 1½-oz (42.5-g) refill tubes.

For Prescribing Information, please see physician package insert.

Mead Johnson LABORATORIES MJL 5-4169
MEAD JOHNSON & COMPANY • EVANSVILLE, INDIANA 47721 USA C-V14-12-85

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
DEPARTMENT OF OBSTETRICS AND GYNECOLOGY

Applications are invited by the Department of Obstetrics and Gynecology for an Assistant Professor position immediately. The applicant should possess a MD degree with laboratory training in experimental pathology, or a PhD degree in experimental pathology; experience in research and teaching required in general gynecologic investigations with strong emphasis in gynecologic oncology, pathology, cytology, tissue and serum markers. The applicant should possess substantial background in carrying out independent research, in coordinating multidisciplinary programs, and in supporting projects with residents and fellows in the Department. Please submit complete curriculum vitae and three letters of references to: Hugh M. Shingleton, MD, J. Marion Sims, Professor and Chairman, Department of Obstetrics and Gynecology, University of Alabama at Birmingham, Birmingham, Alabama 35294. The University of Alabama at Birmingham is an Affirmative Action/Equal Opportunity Employer.

POSITION AVAILABLE REPRODUCTIVE ENDOCRINOLOGIST

The Knoxville unit of the University of Tennessee's College of Medicine, Department of Obstetrics and Gynecology, seeks Board Eligible or Board Certified Reproductive Endocrinologists at Assistant Professor, tenure track level (higher level possible, depending on outstanding experience).

Successful candidate will have University funding for resident teaching, with ample opportunities for private practice development. Expansive practice opportunity developed and awaiting appropriate candidate. Experienced microsurgeon and interest in continuing development on an in-vitro fertilization program required. Opportunities available for scientific and clinical research related to reproductive endocrinology.

Interested persons should send curriculum vitae to:

Chairman, Department of Obstetrics and Gynecology,
University of Tennessee Medical Center
1924 Alcoa Highway, U-27
Knoxville, TN 37920
For more information call (615) 544-9306

An Affirmative Action/Title IX/Section 504 Employer

POSITIONS AVAILABLE

Two full-time positions are available for gynecologic oncologists in the Department of OB/GYN, U.C. Davis Medical Center, Sacramento, California. Applicants should be board certified in OB/GYN and knowledgeable in the areas of chemotherapy and radiation therapy. Applicants must demonstrate experience and competence in teaching and research related activities.

One position, for Associate/Assistant Professor requires the applicant to have successfully completed subspecialty training in gynecologic oncology, with subsequent specialty board eligibility and/or certification.

The second position, for Assistant Professor, requires the applicant to have completed subspecialty training in an approved program in gynecologic oncology.

Responsibilities include patient case management, surgery specific to gynecologic oncology, participation in teaching and development and/or participation in research activities and involvement in University and Hospital committees.

Send curriculum vitae and five references to:

Edward J. Quilligan, M.D.
Chairman, Department of Obstetrics and Gynecology
University of California, Davis, Medical Center
1621 Alhambra Boulevard, Suite 2500
Sacramento, California 95816

The final date for receipt of applications is "Open until filled, but not later than May 15, 1987."

The University of California is an equal opportunity, affirmative action employer.

WASHINGTON STATE MULTISPECIALTY GROUP PRACTICE

The Wenatchee Valley Clinic, a multispecialty group practice of 105 physicians seeks a Board Eligible/Board Certified OB/GYN physician to join its Moses Lake, Washington facility.

Moses Lake is in Central Washington State with close proximity to the Columbia River and the Cascade Mountain Range. The area is ideal for a family-oriented physician interested in the four-season recreational opportunities available.

Excellent professional environment with young, progressive group. Competitive salary and benefit package.

If interested submit CV to: E. Michael Graham, M.D., Moses Lake Branch of Wenatchee Valley Clinic, 840 E. Hill Ave., Moses Lake, Washington 98837.

TRI-LEVLEN

Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

A LOW-DOSE TRIPHASIC OC WITH THE CONFIDENCE THAT COMES FROM EXPERIENCE



*Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. The physician should remain alert to the earliest manifestations of any symptom of serious disease and discontinue oral contraceptive therapy when appropriate. These d represent only the results of selected studies. Therefore, before prescribing, the physician should read and remain alert to all data found in the complete prescribing information for the product. Please also see brief summary of prescribing information on the last page of advertisement.

A formulation with the lowest total hormonal dose in a combination OC

Phased doses of both levonorgestrel and ethinyl estradiol

Permits a decrease in total steroid dose without compromising effectiveness.

Low incidence of breakthrough bleeding

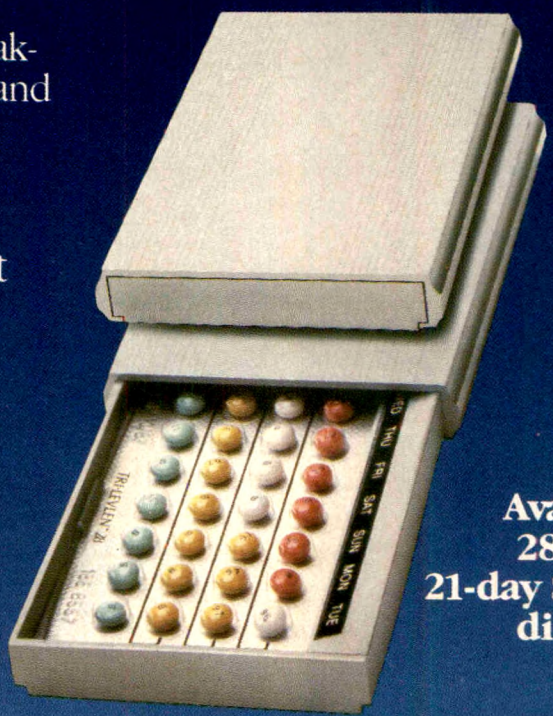
A low 5.7% overall incidence of breakthrough bleeding based on 77 U.S. and U.K. studies involving 3,546 women (35,036 total cycles).[1]

Simple first dose/first day start

The first dose is taken on the first day of menstruation. No need to count days or wait for Sunday starts.

Minimal impact on the following metabolic and hemodynamic parameters*

Lipids;[2,3] carbohydrate metabolism[2,3] and blood pressure[1] were not significantly affected in certain specific studies.



A formulation with the confidence of 170 million cycles of experience worldwide

Available in 28-day and 21-day Slidecase™ dispensers

TRI·LEVLEN™28 TRI·LEVLEN™21

BERLEX™ **Dedicated to innovation in OB/GYN medicine**

TRI·LEVLEN™ 28
TRI·LEVLEN™ 21

Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

BRIEF SUMMARY

Tri-Levlen™—6 brown tablets, each containing 0.050 mg of levonorgestrel (d(-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxy-gon-4-en-3-one), a totally synthetic progestogen, and 0.030 mg of ethinyl estradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3, 17-diol); 5 white tablets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol; 10 light-yellow tablets, each containing 0.125 mg levonorgestrel and 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day triphasic regimen).

Indications and Usage—Tri-Levlen Tablets are indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OCs) as a method of contraception.

Contraindications—OCs should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during the use of OCs or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thrombophlebitis and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to-six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions:* There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.

4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, an increased risk was apparent between 6 and 12 months of use.

7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. Three studies have been performed with the Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alterations in lipid metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.

9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.

10. *Bleeding Irregularities:* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding:* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See "Warnings" section for information on the carcinogenic potential of OCs.

Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."

Nursing Mothers—See "Warnings."

Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted.: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is first taken later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information and package insert.

References: 1. Data submitted to FDA. **2.** Skouby SO, Kühl C, Mølsted-Pedersen L, et al: *Am J Obstet Gynecol* 1985;153:495-500. **3.** Vermeulen A, Thiery M: *Contraception* 1982;26:505-513.

BERLEX™
Dedicated to innovation in OB/GYN medicine

Electromyographic observations on the human cervix during labor

Marjan Pajntar, M.D., Sc.D., Egidija Roškar, Sc.D., and Drago Rudel, M.Sc.
Kranj and Ljubljana, Yugoslavia

The activity of smooth muscles in the cervix is one of the relevant factors for its dilatation during labor, but in humans it has not yet been sufficiently studied. Muscular activity may be observed by measuring electromyographic phenomena. In 60 parturient women of different parity and with various degrees of cervical ripeness at the onset of labor, the cervical electromyographic activity was measured through the entire course of labor in synchronization with uterine pressure measurements and also in 30 cases in synchronization with measurements of the uterine corpus electromyographic activity. The conditions necessary for successful measurements are described. The intensity of the cervical electromyographic activity was found to decrease with the level of cervical ripeness and with parity. When measured in the longitudinal direction, the cervical electromyographic activity resembled that of the uterine corpus, but when picked up from the circular lead, in a few cases of unripe cervices it differed from the electromyographic activity of the uterine corpus. (Am J Obstet Gynecol 1987;156:691-7.)

Key words: Electromyography, cervix in labor

The opinion that the cervix is passive during labor and that its dilatation is due to contraction of the uterine corpus has lately been contested by a number of new findings. The latter pertain to histologic and biochemical changes in the cervix which make possible its efficient dilatation during labor,[1] as well as to the activity of cervical smooth muscles in mammals. The smooth muscles of the ewe's cervix contract vigorously even late in gestation and with the approach of parturition.[2] The cervical and uterine smooth musculatures appear to act independently, possibly reflecting their independent functioning.[2] In humans the factors responsible for efficient cervical dilatation during labor are independent of the factors responsible for efficient uterine contraction.[3]

Material and methods

The study involved 60 parturient women of various parity who were sent to the hospital for induced labor and therefore fulfilled the criteria for programmed labor.[4] In each patient the ripeness of the cervix was estimated according to the Bishop scores; after that an amniotomy was performed, a catheter for measuring intrauterine pressure was inserted, electrodes for detecting the electrical activities of the cervix and uterine corpus were attached, and within 30 minutes an oxytocin solution (Syntocinon) was administered by the drip method in a dose of 6.75 mEq/min. In a few exceptional cases Syntocinon was administered only later in the course of labor. "Partograms" were drawn in all the cases.

In the first 20 labors the detection of the cervical electromyographic activity was unipolar, in the 30 subsequent cases it was bipolar and single-lead (usually in the longitudinal direction), and in the last 10 cases both leads were used, a longitudinal and a circular one. The two-lead configuration is shown in Fig. 1. In 30 labors, a simultaneous bipolar recording of the electrical activity of the uterine corpus was also performed.

Surface Ag-AgCl disc electrodes were used to measure the abdominal uterine corpus electrical activity. They were attached 6 to 7 cm apart above the right or left horn of the uterus, with a reference ground electrode placed between the active electrodes. To obtain the electromyographic activity in the cervix, spiral electrodes, usually used to pick up fetal electromyographic activity, were adapted. These electrodes are standard accessories of the Hewlett-Packard HP 8030A cardiotocographic measuring set. A miniature two-channel differential electromyographic preamplifier was designed to amplify low-amplitude electromyographic potentials with the fixed gain of 1000 and a band width of 0.1 to 5 kHz (3 db). To diminish artifacts caused by body movements during labor, it was fastened to the upper part of a thigh by a belt to permit use of electrode wires that were as short as possible.

Electromyographic signals were further amplified by

From the Hospital of Obstetrics and Gynecology and the Medical Faculty and Faculty of Electrical Engineering, Edvard Kardelj University.
The research study was supported by the Slovenian Research Community, Ljubljana, Yugoslavia.
Received for publication September 24, 1985; revised July 29, 1986; accepted September 26, 1986.
Reprint requests: Prof. Marjan Pajntar, Hospital of Obstetrics and Gynecology, Kidričeva 38 a, 64000 Kranj, Yugoslavia.

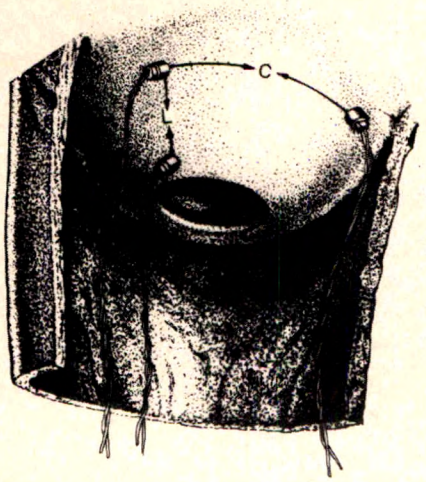


Fig. 1. Position of the spiral electrodes measuring electromyographic activity on the cervix. *L*, Longitudinal lead; *C*, circular lead.

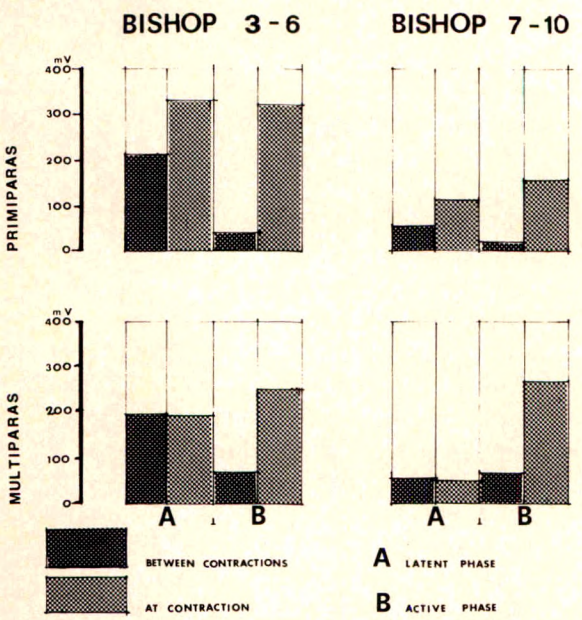


Fig. 2. Mean values of the peak-to-peak amplitude of the electromyographic signal.

a two-channel amplifier with an adjustable gain, an adjustable DC level, and a built-in low-pass filter (5 Hz). Electromyographic potentials were then recorded by a two-channel paper chart recorder which was a part of the above-mentioned cardiotocograph (1 minute = 10 mm). An adequate interface was made which enabled different combinations of simultaneous two-channel registrations.

Intrauterine pressure measurements were performed by means of an open-end saline-filled catheter and a pressure transducer.

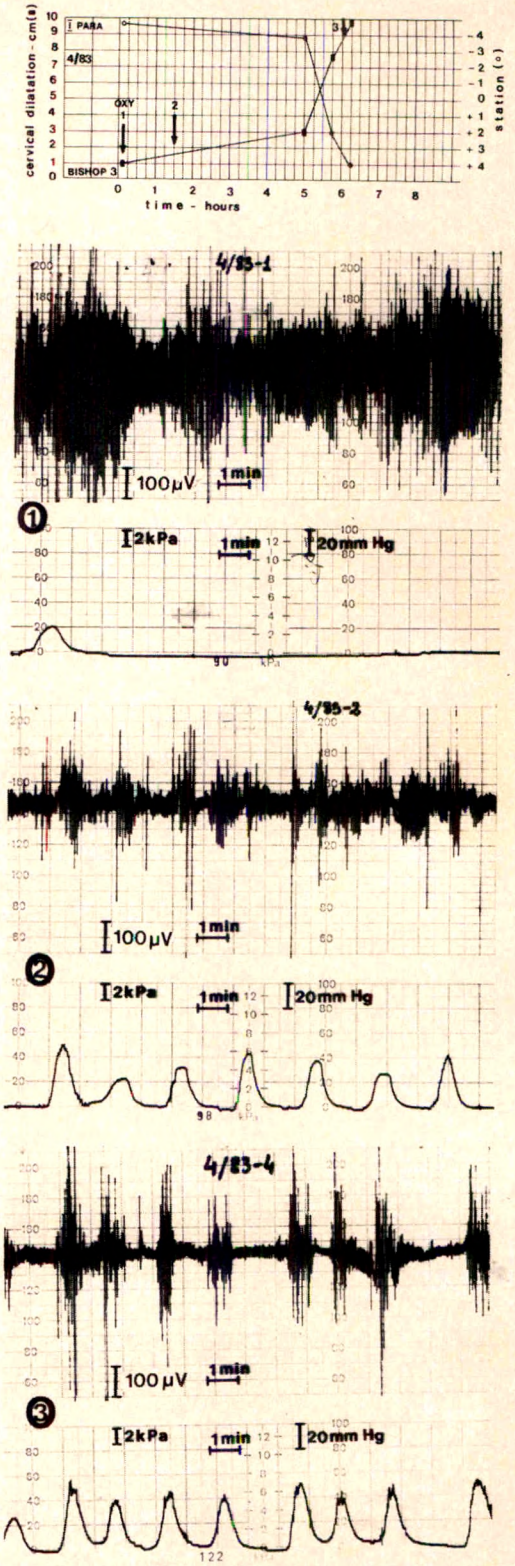


Fig. 3. Partogram, cervical electromyographic activity, and intrauterine pressure in a primiparous patient with unripe cervix. Parts *1*, *2*, and *3* correspond to the events marked in the partogram. For all parts, the upper trace = electromyographic activity and the lower trace = intrauterine pressure ("the basal tone" manually zeroed).

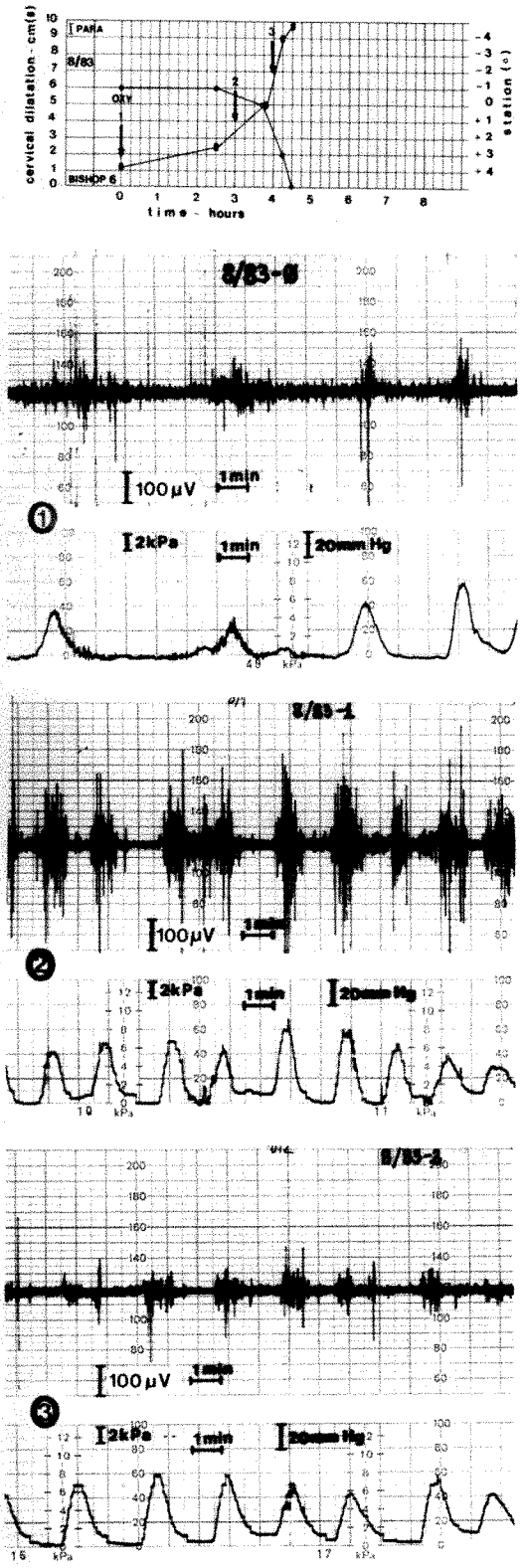


Fig. 4. Partogram, cervical electromyographic activity, and intrauterine pressure of a primiparous patient. Parts *1*, *2*, and *3* correspond to the events marked in the partogram. For all parts, the upper trace = electromyographic activity and the lower trace = intrauterine pressure.

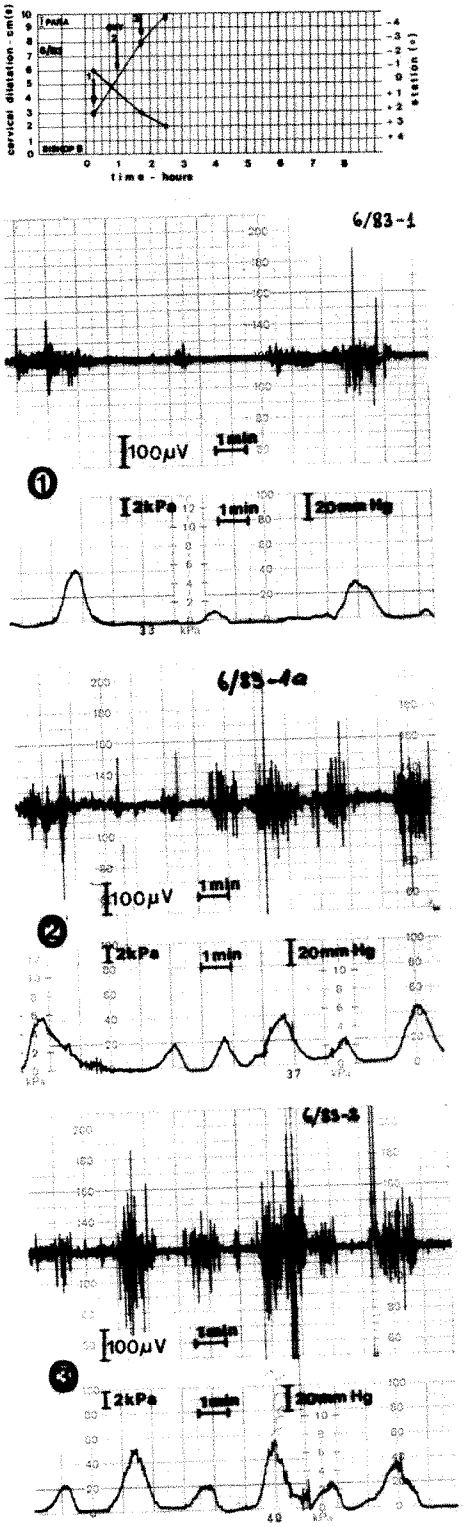


Fig. 5. Partogram, cervical electromyographic activity, and intrauterine pressure of a primiparous patient with ripe cervix. For all parts, the upper trace = electromyographic activity and the lower trace = intrauterine pressure.

Results

Forty-nine appropriate cervical electromyographic recordings were analyzed. They were performed in 28 primiparous women (23 with unripe cervices and 5 with ripe cervices) and in 21 multiparous women (10 with unripe cervices and 11 with ripe cervices). The peak-to-peak amplitude of the strongest burst of electromyographic signal was measured at a uterine contraction and between two contractions in the initial phase of labor—immediately after amniotomy and in the active phase after cervical dilatation of >6 cm. The mean values for each group are presented in Fig. 2.

In an attempt to describe the observed phenomena, typical segments of electromyographic recordings are presented below.

Ripeness of the cervix. The less ripe the cervix, the more intensive is its electrical activity during induced labor immediately after amniotomy (Figs. 3 to 5, *part 1*).

In unripe cervices a strong permanent activity is recorded at the beginning of the latent phase of dilatation, without any noticeable contractions of the uterine corpus (Fig. 3, *part 1*). This activity is subsequently grouped into increasingly pronounced bursts, which are usually synchronized with uterine contractions, but occasionally they also appear between two contractions (Fig. 3, *part 2*). In this period the activity occurring between two contractions is stronger than later in the active phase of dilatation. In riper cervices the initial activity is smaller and grouped into bursts, which occur in synchronization with uterine contractions but occasionally somewhat earlier or later (Figs. 4 and 5, *part 1*). In the active phase of cervical dilatation the activity appears to be grouped in bursts, usually in synchronization with uterine contractions and persisting throughout a contraction. The intensity changes in the course of labor. These changes are not always correlated to the intensity of uterine contractions (Fig. 4, *parts 2* and *3*). The activity, which is ungrouped at the onset of labor, groups and intensifies after administration of the oxytocin preparation. Weak electrical activity associated with infrequent contractions (Fig. 5, *part 1*) increases immediately after administration of oxytocin, and the frequency of contractions increases (Fig. 5, *parts 2* and *3*).

Parity. In principle, the intensity of the cervical electrical activity decreases with parity. Even in a patient who is para 2 with an extremely unripe cervix, electrical activity was observed similar to that found in primiparous patients with unripe cervices. In most cases, however, in secundiparous patients the electrical activity of the cervix bears more resemblance to that characteristic of primiparous women with ripe cervices (Fig. 5), although the cervical dilatation may be slower than in primiparous women with ripe cervices. In triparous and multiparous women the activity is smaller. As a rule, the amplitudes of individual bursts in multiparous women are much smaller. This type of minimal activity is exemplified here by the case of a triparous patient with a cerclage performed during pregnancy because of cervical insufficiency (Fig. 6). After oxytocin had been administered, uterine contractions became more frequent and stronger (Fig. 6, *parts 2* and *3*). The electrical activity appeared to be partly grouped in bursts of small amplitudes. The bursts occurred simultaneously with contractions as well as between two contractions, mostly with a nonuniform waveform and sequence, throughout the labor.

Electromyographic activity of the cervix and uterine corpus. The electromyographic activity of the uterine corpus was observed and compared to the electromyographic activity of the cervix. Because of the different types of measuring techniques implemented, the amplitude proportions between the two groups of results are of course only relative. The cervical electromyographic detection was unipolar and bipolar. After amniotomy the electrical activity in unripe cervices is usually greater than that in the corpus, whereas in the case of ripe cervices the situation is reversed. Later during labor, especially in the active phase of dilatation, the proportion between the two changes independently of the intensity of contractions. In the final phase of dilatation the intensity of electromyographic activity is usually smaller in the cervix than in the corpus (Fig. 7, *part b*). The cervical and uterine corpus electromyographic activities occur in groups of bursts, which are in most cases synchronized both with each other and with uterine contractions. In a few cases, however, the distribution of bursts along the time scale in the cervix is entirely different from that in the corpus (Fig. 7, *part a*).

Two-lead detection of the cervical electromyographic activity. The recording of the cervical electromyographic activity measured longitudinally and circularly are in most cases similar. However, in a few cases the electromyographic activity detected from the two leads differs, for example, in a primiparous woman with unripe cervix, Bishop score of 3 (Fig. 8). The differences are significant, especially in the latent phase of cervical dilatation (Fig. 8, *parts a* and *b*), whereas in the active phase of dilatation they diminish (Fig. 8, *parts c* and *d*). Immediately after amniotomy (Fig. 8, *part a*) the longitudinal activity is minimal. The circularly measured activity is stronger and is manifested as bursts of shorter or longer duration. After oxytocin has been administered (Fig. 8, *part b*), the longitudinal electromyographic activity intensifies. The increase is even greater in the circular direction. It is manifested in

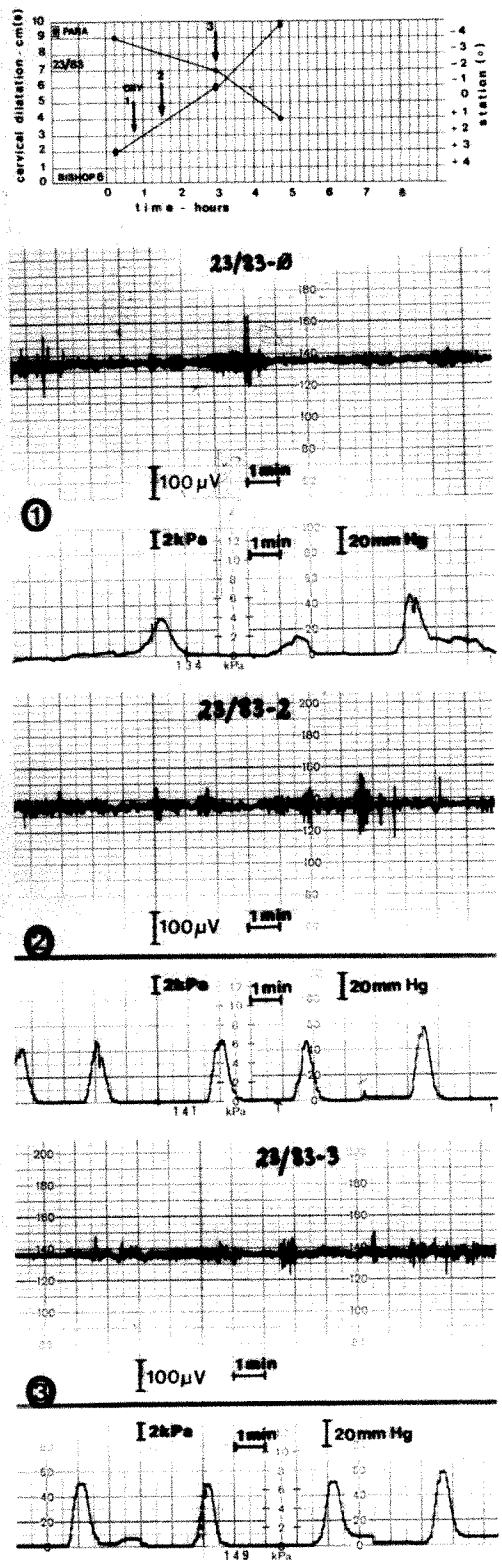


Fig. 6. Partogram, cervical electromyographic activity, and intrauterine pressure of a multiparous patient. Parts *1*, *2*, and *3* correspond to the events marked in the partogram. For all parts, the upper trace = electromyographic activity and the lower trace = intrauterine pressure.

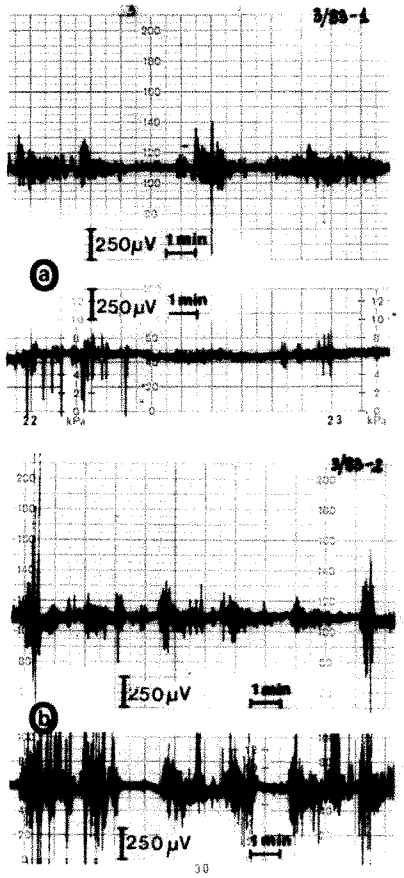


Fig. 7. Comparison of cervical and uterine corpus electromyographic activity measured at the beginning *(a)* and in the middle *(b)* of cervical dilatation in a primiparous patient. The upper trace = electromyographic activity of the cervix and the lower trace = electromyographic activity of the uterine corpus.

different shapes. The activity measured from both leads rapidly increases at the beginning of the active phase of dilatation (Fig. 8, *part c*). The longitudinal activity is relatively well grouped and synchronized with uterine contractions. The circular activity, however, is less well grouped. In the final phase of dilatation (Fig. 8, *part d*) a decrease in both activities is evident, particularly in the circular direction. At this stage the similarity and synchronization between the two activities is evident.

Comment

Adequate cervical dilatation is an important factor for a successful course of labor. The results of the measurements of the cervical electromyographic activity during labor confirm that also in the human the cervix is not just a passive part of the uterus but that its muscles also actively contract.

The results obtained so far primarily confirm the presence of electromyographic activity in the cervix

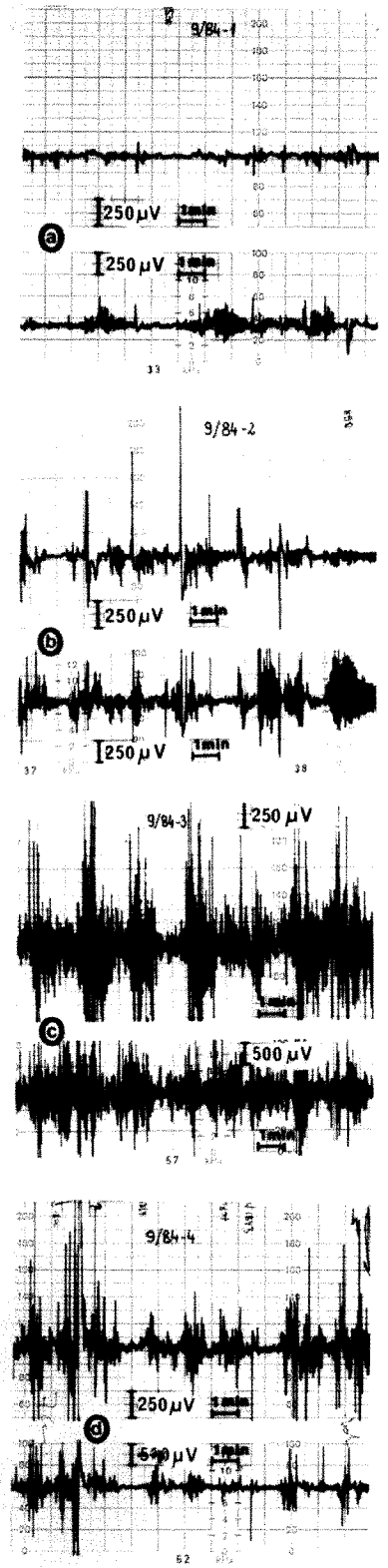


Fig. 8. Comparison of the cervical electromyographic activity measured from the longitudinal lead *(upper trace)* and from the circular lead *(lower trace)*. Part *a* = soon after amniotomy; part *b* = after oxytocin infusion; part *c* = at the 3 cm cervical dilatation; part *d* = final phase of dilatation.

during labor and establish the relevant conditions that have to be fulfilled to achieve efficient and high-quality recordings of this activity. They also suggest that this electromyographic activity appears in two directions, longitudinally and circularly.

The spiral electrodes used in our study to detect the cervical electromyographic activity served our needs well. It is important that they are firmly fixed in the tissue, which of course requires a certain amount of experience. In our first attempts the electromyographic recordings tended to be unreliable and of poor quality, especially toward the end of labor when the women grew restless and artifacts occurred because of movement of the electrodes in the tissues.

Another parameter tested in our investigation was the type of detection, which may be unipolar or bipolar. When unipolar detection was used, far more artifacts were found in the recordings. That is understandable, since such detection picks up the electrical potentials between the measuring electrode and the reference one and covers the potentials generated not only by the cervical muscles but also by all the structures between the two electrodes.

On the basis of the above findings resulting from our preliminary measurements, it was finally decided to use bipolar detection in our further work, since it yields the electrical activity between the two active electrodes inserted in the cervix.

At the initial stage of bipolar measurements of the cervical electromyographic activity, little attention was paid to positioning of the electrodes in terms of discriminating the electrical activity in the respective longitudinal and circular direction. As the difference between the electromyographic activity of the uterine corpus and that of the cervix was noticed, however, it led to the conclusion there might be differences in the electrical activity resulting from the use of different leads as well as from the activity of differently positioned muscle fibers (in the longitudinal and circular direction, respectively).

It was found that, in addition to the regular activity in the longitudinal direction, another, different activity in the circular direction occurs as well.

Because of the above-described course of our investigation, in most of our experimental cases the electrical activity of the longitudinal fibers of smooth cervical muscles was recorded. Their activity resembles that of the uterine corpus. This fact leads to the conclusion that these fibers may be excited by stimuli spreading downward from the corpus. The active contraction of the longitudinal fibers probably helps to shorten, open, and retract the cervix.

Our observations of the longitudinal electromyographic activity in the cervix agree with the findings of

some other authors[5] regarding the activity of smooth muscles in the uterine corpus: initially it is more nonuniform, and subsequently it groups into so-called bursts. The difference between the uterine corpus and the cervix activity exists in the fact that the electromyographic activity in the corpus normally anticipates the increase of the intrauterine pressure, whereas in the cervix it appears at the onset of contractions and persists throughout an entire contraction. The intensity of this activity varies in the course of labor quite independently of the intensity of the electromyographic activity of the uterine corpus and of the intensity of contractions. We therefore believe it is partly independent of the uterine corpus.

Particularly interesting is the circular electrical activity in the cervix, measured with the circular lead. This activity is entirely different from that found when recording from the longitudinal lead. It usually occurs in unripe cervices and in primiparous women at the beginning of labor and probably represents the circular muscle fiber activity. At present it can only be said that in all probability this activity does not exist in every woman, but that it is very important for the cervical tonus at the beginning of labor and that later it hinders effective dilatation of the cervical canal.

Visual evaluation of recorded electromyographic activity without more subtle subsequent analyses does not permit more precise description of the electrical phenomena in the cervix during labor. Recent advances in computer analyses of bioelectrical signals make their quantitative explanation possible. Spectral analysis has been implemented for this purpose, and the changes in typical frequencies in the power density spectral function of the cervical electromyographic signal were studied. The results are encouraging in helping to differentiate the contribution of the two types of muscle fibers in the cervix to its efficient dilatation during labor. It is hoped that we will be able to identify the differences between the electromyographic activity of the cervix and that of the uterine corpus.

REFERENCES

1. Turnbull AC, Anderson BM. Uterine function in human pregnancy and labour. In: MacDonald RR, ed. Scientific basic of obstetrics and gynecology. New York: Churchill Livingstone, 1978;79-108.
2. Stys Sj, Clewell WH, Meschia G. Changes in cervical compliance at parturition independent of uterine activity. AM J OBSTET GYNECOL 1978;130:414-8.
3. Pajntar M, Čuk M, Rojšek J, Rizner T. A factor system of the induced first stage of labour [Research report]. Kranj, Yugoslavia: Research Foundation of Slovenia, Yugoslavia, 1977:1-52.
4. Pajntar M, Čuk M, Simoniti H. Programmed versus spontaneous and stimulated labour. Z Geburtshilfe Perinatol 1977;181:174-7.
5. Wolfs GM, Leeuwen MV. Electromyographic observations on the human uterus during labour. Acta Obstet Gynecol Scand [Suppl]1979;90:1-62.
6. Roškar E, Pajntar M, Rudel D. Signal processing of human parturient uterine corpus and cervic electromyography: a new approach to quantified clinical monitoring of labour. Med Biol Eng Comput 1985;23(suppl):940-1.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1987 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 156 (January-June) and Vol. 157 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Significant bacteremia associated with replacement of intrauterine contraceptive device

Sarah Murray, B.M., J. B. Hickey, M.A., B.M., B.Ch., and Elizabeth Houang, M.B., Ch.B.
London, England

Blood culture samples were taken from 23 women at different stages when an intrauterine contraceptive device was replaced. Transient bacteremia resulting from vaginal organisms was found in 13% of women 4 to 6 minutes after insertion of the new device. Previous reports have failed to demonstrate bacteremia associated with either first insertion or removal of intrauterine contraceptive device. Our results show that replacement of an intrauterine contraceptive device, a more traumatic procedure, causes significant bacteremia, and chemoprophylaxis should be given to patients in the groups at risk. (AM J OBSTET GYNECOL 1987;156:698-700.)

Key words: Bacteremia, vaginal; intrauterine contraceptive device; chemoprophylaxis

The process of inserting an intrauterine contraceptive device involves intracervical instrumentation, which might be expected to introduce vaginal bacteria into the uterine cavity and provide a source for potential bacteremia. Major complications after such a procedure have been reported in certain high-risk groups of patients; these include bacterial endocarditis in patients with valvular heart disease and overwhelming septicemia in immunosuppressed patients.[1, 2] It has therefore been recommended that patients with a prosthetic heart valve should receive antibiotic prophylaxis for the insertion or removal of an intrauterine contraceptive device.[3]

Despite the theoretical risk of bacteremia, previous studies designed to assess the incidence of its occurrence have failed to demonstrate significant bacterial growth in blood cultures taken after either insertion or removal of the device.[4, 5]

It is now common practice for many women to have their intrauterine contraceptive devices changed at regular intervals by their own general practitioner. We wished to study the incidence of significant bacteremia after the routine replacement of an intrauterine contraceptive device in the doctor's office, so that the potential risk could be assessed and become more widely known among doctors offering this service.

Patients and methods

Patients of one medical practice were studied. These were patients for whom a change of intrauterine contraceptive device was due, and for whom the device continued to provide an appropriate method of contraception. The patient was allocated the replacement intrauterine contraceptive device (Nova T or Gravigard) according to the randomized code.

The old intrauterine contraceptive device was first removed under the vision of a sterile bivalve speculum with the long-handled forceps. The next procedure was a bimanual examination to exclude cervical or adnexal tenderness. The cervix was again exposed and swabbed with a disinfectant (Sartodil, ICI) three times with the aid of a vulsellum applied to the anterior lip of the cervix. The new intrauterine contraceptive device was then inserted according to the technique recommended by the manufacturer. All patients were seen 6 weeks after the replacement of the intrauterine contraceptive device for a check-up appointment. Throughout the procedure, aseptic nontouch technique was used with gloved hands by the operator. The gloves were changed after the bimanual examination.

Blood samples were obtained from an intravenous sampling catheter (Wallace Y Can 2/g) inserted into a forearm vein after the skin was disinfected with 70% isopropyl alcohol. Samples (20 ml) were obtained as control at the start of the procedure, after removal of the old coil, and at approximately 2, 5, 7, and 10 minutes after insertion of the new coil. Sterile normal saline solution, 0.9%, was used to flush the cannula between samples. Each blood sample was immediately injected into a set of blood culture bottles consisting of a Digest Broth with 0.1% glucose and Liquoid (Southern Group Laboratories) and a Fastidious Anaerobic Broth containing thymidine (LAB M). The blood culture bottles were incubated within 3 hours and processed in a routine manner by the microbiology department of the Chelsea Hospital for Women.

From the Chelsea Hospital for Women.
Received for publication May 20, 1986; accepted September 24, 1986.
Reprint requests: Dr. Elizabeth Houang, Department of Microbiology, Chelsea Hospital for Women, Dovehouse Street, London SW3 6LT, England.

Table I. Bacterial isolates from the blood cultures obtained during the replacement of intrauterine contraceptive devices

Patient No.	*Time interval when positive culture was obtained*	*Bacterial species*
1	After removal of Copper-7	*Staphylococcus epidermidis* and nonhemolytic streptococcus
2	After removal of Multiload	Anaerobic diphtheroid
3	7 min after insertion	*Bacteroides* sp.
4	4 min after insertion	*Gardnerella vaginalis*
5	5 min after insertion	*G. vaginalis*
6	10 min after insertion	*S. epidermidis*
7	7 min after insertion	*S. epidermidis* and anaerobic diphtheroid
8	4 min after insertion	*S. epidermidis* and diphtheroids
9	2 min after insertion	Anaerobic diphtheroids

Results

Twenty-three women were studied: 12 received a Gravigard intrauterine contraceptive device and 11 a Nova T device. All new devices remained satisfactory at the follow-up appointment with no evidence of adnexal infection during the bimanual examination.

A total of 138 blood culture sets were examined from the 23 patients. Bacterial growth was observed in 13 blood culture sets from 10 women. All the control blood cultures were negative except for one patient in whose sample bottle *Staphylococcus epidermidis* grew. The details of the positive cultures from the other nine patients are shown in Table I. There was no significant correlations between the likelihood of bacteremia and the parity of the women, the duration of use of the old intrauterine contraceptive device, and the type of the new device.

Comment

Throughout the procedures, nontouch aseptic technique was used with gloved hands. The blood samples were drawn from the intravenous catheter in the forearm either by the nurse not involved in the procedures or by the operator herself with the gloves removed. It is therefore unlikely that the blood samples would be contaminated by organisms from the vagina. Of the bacterial species isolated, *Gardnerella vaginalis* and *Bacteroides* may be assumed to be of vaginal origin, since they are normally found in the vagina but not on the skin, whereas the other species are commonly found from both sites.[6] Thus the incidence of bacteremia of vaginal origin is at least 13% (3/23). All cases occurred transiently 4 to 6 minutes after insertion of the new intrauterine contraceptive device, that is, after three different gynecologic procedures: removal, bimanual examination, and insertion. The summation of these procedures may increase the chance of a positive culture. In contrast, the negative findings reported by other workers were based on blood culture studies performed during the first insertion or removal of an intrauterine contraceptive device. Everett et al.[4] examined aerobic blood cultures obtained before and at various intervals up to 1½ hours after intrauterine contraceptive device insertion in 84 women and after removal of the device in 16 women. They did not find significant bacterial growth other than isolated positive cultures resulting from skin contaminants, although they did not report that anaerobic blood culture studies were performed. These authors therefore concluded that chemoprophylaxis during insertion or removal of an intrauterine device would be unnecessary in the absence of evident pelvic infections. Hall et al.[5] also failed to demonstrate bacteremia in blood cultures obtained from 25 women before and within 3 to 8 minutes of intrauterine contraceptive device insertion.

At the time of intrauterine contraceptive device replacement, none of our patients had symptoms or signs of pelvic infection, which has been reported to occur in 2.5% of patients in the first year of use of the device.[7] All the devices involved in our study were of the tailed type. Sparks et al.[8] demonstrated the presence of a small number of bacteria in the uterine cavity long after insertion of a tailed device. Jacques et al.[9] showed that in experimental rabbits, microorganisms colonized the core surface of both the tailed or untailed devices if they were inserted via the cervix. We did not perform a culture of the removed device, since it was difficult to avoid contamination with vaginal flora during the retrieval procedure. It is therefore uncertain whether bacteremia demonstrated in our study was associated with an infected/colonized device. It is reasonable to assume that the chance of having a positive blood culture would be increased by replacement of the device in an uterus already colonized by vaginal organisms.

Almost half a million women currently use the intrauterine contraceptive device in the United Kingdom, and replacement of the device is becoming a commonly performed procedure, often in the general practice. The risk of bacteremia associated with a first insertion or removal of the device may be very slight.[4, 5] The results of our study, however, show that replacement of the device is associated with bacteremia of vaginal

origin, with an incidence of at least 13%, thus lending support to the cautious attitude expressed by Sparks.[10] If it is deemed necessary on balance to fit an intrauterine contraceptive device in a woman who has prosthetic heart valves, had previous cardiac surgery, or is a drug abuser, antibiotic cover for insertion and replacement should be mandatory and follow-up should be performed at a hospital-based clinic where sterilized instruments are available. An absolute contraindication should be a history of pelvic inflammatory disease or infective endocarditis. To minimize the risks of bacteremia, one should also consider the use of an intrauterine contraceptive device licensed for 4 to 5 years and perhaps carry out the removal and insertion of the device at different clinic appointments.

We thank Schering Pharmaceuticals for the grant to cover the cost of material and transport of specimens.

REFERENCES

1. Smith PA, Ellis CJ, Sparks RA, Guillebaud J. Death associated with intrauterine contraceptive devices in the United Kingdom between 1973 and 1983. Br Med J 1984;28:1537-8.
2. Risks of intrauterine contraceptive devices. Br Med J 1984;288:1554-5.
3. Report of a Working Party of the British Society for Antimicrobial Chemotherapy. The antibiotic prophylaxis of infective endocarditis. Lancet 1982;2:1323-6.
4. Everett ED, Reller LB, Droegemueller W, Greer BE. Absence of bacteremia after insertion or removal of intrauterine device. Obstet Gynaecol 1976;47:207-9.
5. Hall SM, Jamieson JR, Witcomb MA. Bacteraemia after insertion of intrauterine devices. S Afr Med J 1976; 50:1232.
6. Isenberg HD, D'Amato RF. Indigenous and pathogenic microorganisms of humans. In: Lennette EH, Balows A, Hausler WJ, Jean Shadomy H, eds. Manual of clinical microbiology. 4th ed. Washington DC: American Society for Microbiology, 1985:24-5.
7. Meeker CI. Use of drugs and intrauterine devices for birth control. N Engl J Med 1969;280:1058-60.
8. Sparks RA, Purrier BGA, Watt PJ, Elstein M. Bacterial colonisation of uterine cavity: role of tailed intrauterine contraceptive device. Br Med J 1981;282:1189-91.
9. Jacques M, Loson ME, Costerton JW. Microbial colonization of tailed and tailless intrauterine contraceptive devices: influence of the mode of insertion in the rabbit. Am J Obstet Gynecol 1986;154:648-55.
10. Sparks RA. Endocarditis and the IUD. Br J Fam Plann 1985;11:19-23.

Measurement of antibody to *Mycoplasma hominis* by an enzyme-linked immunoassay and detection of class-specific antibody responses in women with postpartum fever

Mary B. Brown, Ph.D., Gail H. Cassell, Ph.D., William M. McCormack, M.D., and Jerry K. Davis, D.V.M., Ph.D.

Gainesville, Florida, Birmingham, Alabama, and Brooklyn, New York

The standard conditions for detection of human IgG, IgM, and IgA antibodies to *Mycoplasma hominis* by an enzyme-linked immunosorbent assay (ELISA) were established with the use of a cell lysate antigen and alkaline phosphatase conjugates. Antigen was used at a concentration of 10 μg of protein per milliliter, sera were diluted 1:200, and conjugates were diluted 1:500. Agreement between cultured isolation of *M. hominis* from the lower genital tract and presence of antibody in 207 women was 71%, 82%, and 86% for IgG, IgM, and IgA, respectively. When the ELISA was compared with the mycoplasmacidal assay, an overall agreement of 81% occurred, with the majority of the discrepancies occurring in the ELISA-positive and mycoplasmacidal-negative category. A linear relationship between end point titer and the A400 value (ELISA or absorbance value at 400 nm) at a standard serum dilution was demonstrated for the IgG, IgM, and IgA classes. Although the ELISA was relatively independent of antigen heterogeneity, no single strain detected more than 87% of positive sera, thus suggesting that optimum detection of antibody to *M. hominis* by the ELISA will require use of antigen pools derived from multiple strains of *M. hominis*. (Am J Obstet Gynecol 1987;156:701-8.)

Key words: *M. hominis*, ELISA, class-specific antibodies, postpartum fever

Depending on a number of factors, including age, sexual experience, and socioeconomic background, *Mycoplasma hominis* can be isolated from the lower genitourinary tract in up to 53.6% of healthy adults. However, carefully controlled cultural and serologic studies suggest that under certain circumstances this opportunistic microorganism is capable of invading the upper genitourinary tract and is of etiologic significance in postpartum fever, salpingitis and pyelonephritis.[1] *M. hominis* can also cause amniotic fluid infection, meningitis, submandibular adenitis, and subcutaneous abscesses in neonates and has been implicated in congenital pneumonia.[1,2]

The commensal nature of *M. hominis* in normal individuals necessitates that definitive diagnosis of disease must depend not only on cultural isolation but also on appropriate clinical manifestations in conjunction with acute and convalescent serology.[1] A variety of serologic methods have been used for detection of antibody to *M. hominis;* all have technical difficulties. The hemagglutination inhibition test is the most sensitive but is difficult to standardize. The metabolism inhibition assay is fairly easy to perform, but the time at which the assay is read is a major cause of nonreproducibility of results. In addition, it is less sensitive than hemagglutination inhibition, and there is little correlation between the two tests. The mycoplasmacidal test may be more sensitive than metabolism inhibition but sensitivity and specificity are affected by many factors, including antigen concentrations and complement activity.

The enzyme-linked immunosorbent assay (ELISA) has been applied to the detection of antibodies against other mycoplasma species and has been found to be a rapid, reliable, and sensitive means of diagnosis. The objectives of the present study were to establish an ELISA for detection of class-specific antibody to *M. hominis* and to compare its efficacy to that of an established serologic assay for detection of antibody responses. Because of the documented etiologic significance of *M. hominis* in postpartum fever, comparisons were made with the use of sera from patients with postpartum fever.

Material and methods

Mycoplasma strains. Reference strains of *M. hominis* PG21 and *M. pneumoniae* FH-Liu were obtained from

From the Department of Infectious Diseases, University of Florida, the Departments of Microbiology, Biology, and Comparative Medicine, University of Alabama in Birmingham, and the Division of Infectious Diseases, Downstate Medical Center.

Supported by Research Grants HD 11447 and HD 16199 to Dr. Gail H. Cassell from the National Institute of Child Health and Human Development and P60 AM 20614 to Dr. Gail H. Cassell from the National Institute of Arthritis, Metabolism, and Digestive Diseases.

Received for publication April 1, 1986; revised September 11, 1986; accepted October 16, 1986.

Reprint requests: Dr. G. H. Cassell, Department of Microbiology, University of Alabama in Birmingham, University Station, Birmingham, AL 35294.

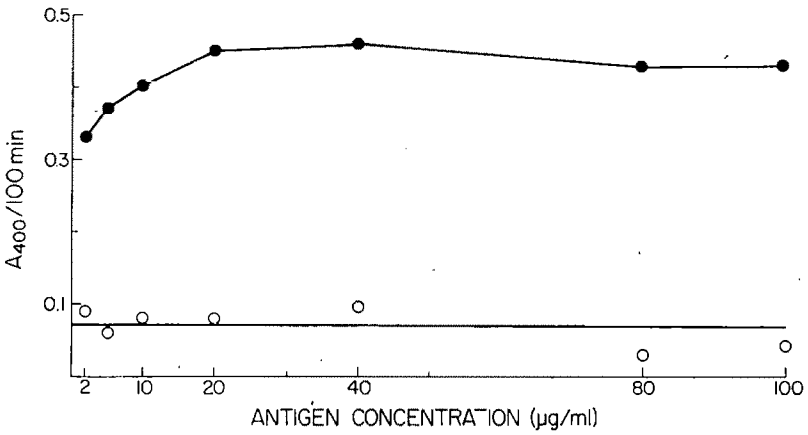


Fig. 1. Effect of *M. hominis* PG21 antigen concentration. Tubes were coated 4 hours, at 37° C, and evaluated with human immune (●) and normal sera (○) diluted 1:200. Anti-IgG conjugate was diluted 1:500.

Table I. Standard ELISA conditions for detection of human antibodies to *Mycoplasma hominis*

ELISA reagent	*Concentration**	*Diluent†*	*Incubation time*	*Incubation temperature*
Antigen	10 μg protein/ml	CBB	4 hr	37° C
Serum	1:200	PBSTA	5 hr	Room temperature
Conjugate	1:500 (IgG, IgM), 1:250 (IgA)	PBSTA	Overnight	Room temperature
Substrate	1 mg/ml‡	SB	30 min (IgG), 1 hr (IgM, IgA)	37° C

*All concentrations are dependent on lot number of conjugate and antigen used and should be reevaluated for each new lot of reagent.

†CBB = 0.1 mol/L sodium carbonate buffer, pH 9.6; PBSTA = phosphate-buffered saline with 0.05% Tween 20 and 0.02% sodium azide; SB = 0.05 mol/L sodium carbonate buffer, pH 9.8, with 0.001 mol/L magnesium chloride.

‡Alkaline phosphatase substrate tablets (Sigma Chemical Company, St. Louis, Missouri).

Research Resources Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland. Six additional strains of *M. hominis* were obtained from J. S. Lin, Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, and have been described previously.[3, 4] Serotypes 1 to 8 of *Ureaplasma urealyticum* were obtained from E. A. Freundt, Institute of Medical Microbiology, University of Aarhus, Aarhus, Denmark. Glucose fermenters were grown in basal liquid medium, while non–glucose fermenters were cultivated in Shepard's liquid 10B medium supplemented with arginine (0.2% final concentration).

ELISA antigens. Ten milliliters of each microorganism in logarithmic growth (12 to 20 hours, 10^5 to 10^8 cfu/ml, dependent on mycoplasma strain) was diluted in broth (1:15 to 1:20) and harvested by centrifugation at 10,000 × g for 30 minutes at 4° C after a color change (12 to 20 hours). The pellet was resuspended and washed three times with sterile phosphate-buffered saline, pH 7.3. Bacterial contamination was monitored by inoculation of tryptic soy and blood agar plates. The washed pellet of cells was resuspended in phosphate-buffered saline (5 mg of protein per milliliter) and lysed by addition of 0.05 mol/L carbonate-bicarbonate buffer, pH 10.0, at 37° C, as described by Horowitz and Cassell.[5] The protein content of each lysate was determined, and all antigens were stored at −70° C.

Standard human reference sera. The human "immune" standard used was a pool of 15 sera from individuals known to be serologically positive in either the metabolism inhibition or mycoplasmacidal assay for *M. hominis* and culturally positive for *M. hominis* in the lower genital tract (cervix-vagina). The normal human serum used in the standardization assays was a single serum available in large quantity with an ELISA value <1 SD from the mean of 40 sera obtained from culturally negative individuals.

Clinical serum samples. Paired acute and convalescent phase sera from 11 women and five unpaired serum samples (one acute, four convalescent) from patients with postpartum fever of documented mycoplasmal origin that have been described previously[6, 7] were analyzed. Serum samples were collected at the time of delivery and 4 to 6 weeks post partum. Sera were heat inactivated at 56° C for 30 minutes and stored at −20° C. The antibody titers in these 27 sera were determined by J. S. Lin with the complement-depen-

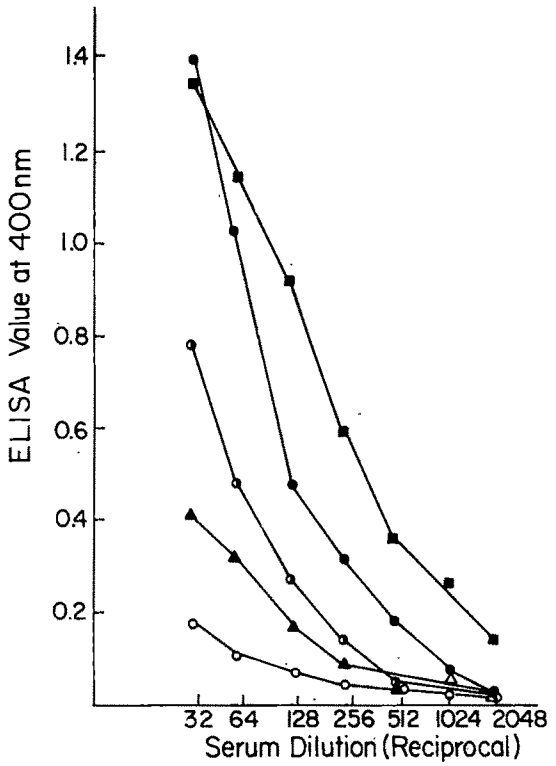


Fig. 2. Effect of serum dilution on detection of IgG in positive (●, ■, ◑, ▲) and negative (○) sera.

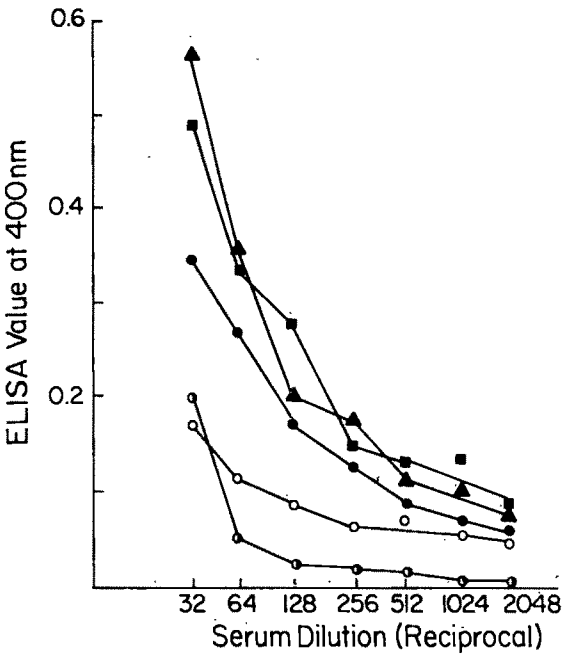


Fig. 3. Effect of serum dilution on detection of IgM in positive (▲, ■, ●) and negative (◑, ○) sera.

dent mycoplasmacidal test[4] and some have been previously reported.[7]

For comparative purposes, acute and convalescent serum pairs from six patients with nongonococcal urethritis of presumed ureaplasmal origin[8] were obtained from M. C. Shepard, Camp LeJeune Naval Hospital, Jacksonville, North Carolina.

In order to determine correlation between serology and culture, serum and cervicovaginal cultures were obtained as described previously[9] from 207 women undergoing laparoscopy for diagnosis of infertility (169 patients) or laparoscopy for tubal ligation (38 patients).

Four reference sera with known complement fixation titers to *M. pneumoniae* were provided by the Alabama State Board of Health Laboratory, Montgomery, Alabama.

Enzyme-conjugated anti-immunoglobulins. Alkaline phosphatase conjugates (Flow Laboratories) to human heavy γ-chain, μ-chain, and α-chain were used to detect the IgG, IgM, and IgA response, respectively. Conjugates were stored at 4° C and diluted immediately before use.

Detection of antibody. Incubation times and concentrations of antigen, conjugate, and serum were determined by checkerboard titration as described previously.[8] Before each addition, antigen-coated tubes were washed three times with 0.9% sodium chloride containing 0.05% Tween 20. All additions were in 1 ml volume. A background control in which phosphate-buffered saline containing 0.05% Tween 20 and 0.02% sodium azide was substituted for serum was included in each assay. Initially, a control tube coated with sterile 10B medium was included to test for nonspecific reaction to medium components, including reactions to yeast extract and horse serum. This control was always negative and was excluded from later assays. The reaction was stopped by addition of 0.1 ml of 1N sodium hydroxide and the absorbance determined at 400 nm.

End point titer analysis. Sera from randomly selected patients with postpartum fever were diluted twofold and tested in the ELISA. The reciprocal of the last dilution to give an A400 value >0.1 was deemed the end point titer. The end point titer versus the A400 value (absorbance value at 400 nm) at the 1:128 dilution was plotted to give a standard regression line.

Statistical analysis. Results were analyzed with a computer-assisted statistical analysis system. The Spearman rank order correlation coefficient, Pearson product moment correlation coefficient, Kruskal-Wallis test, and χ^2 test for independence with Yates' correction factor were used where appropriate.

Results

Standard assay conditions. The standard conditions established by checkerboard titration and chosen for the ELISA are given in Table I. The optimum antigen concentration for detection of IgG antibodies against *M. hominis* was 20 μg of protein per milliliter (Fig. 1).

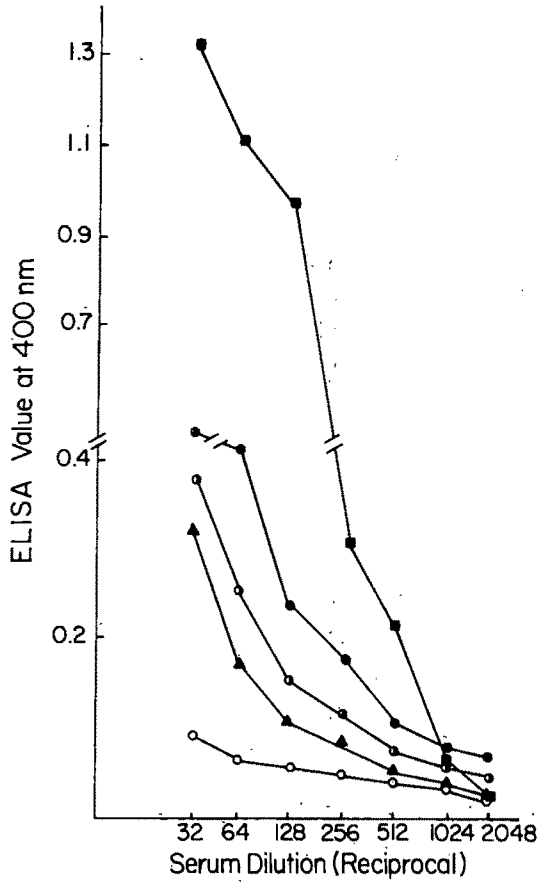


Fig. 4. Effect of serum dilution on detection of IgA in positive (■, ●, ◑, ▲) and negative (○) sera.

Similar results were found for IgM and IgA (data not shown). Since lower concentrations of antigen gave adequate differentiation between human immune and normal human serum standards, 10 μg of protein per milliliter was chosen to conserve antigen and to avoid problems associated with oversaturation of binding sites.[10] The effect of serum dilution at the standard antigen and conjugate concentration is shown for IgG (Fig. 2), IgM (Fig. 3), and IgA (Fig. 4). The serum dilution of 1:200 was selected as the standard dilution since it appeared to be near the midpoint of the curves for all three antibody classes.

Assay reproducibility. Twenty-nine samples were assayed twice (6 weeks apart) with the use of IgG conjugate with a correlation coefficient of 0.939 ($p < 0.001$). In addition, the SD of the normal human serum and human immune standards was determined on the basis of a minimum of 12 assays during a 5-month period. The SD for normal human serum was 0.038 with an SE of 0.01, and for human immune, 0.114 with an SE of 0.03. Similar results were obtained for the IgM and IgA classes.

Assay specificity. Human reference sera with known complement fixation titers (range 32 to 128) to *M. pneumoniae* did not react with the *M. hominis* ELISA antigen (<0.13 optical density). All *M. pneumoniae* reference sera reacted in the ELISA (>0.300 optical density) with *M. pneumoniae* antigen.

Because *M. hominis* is frequently coisolated with *U. urealyticum*, sera from 207 women were assayed in both the *M. hominis* and *U. urealyticum*[8] ELISAs to determine if any correlation in ELISA levels occurred. Individuals who were infected with ureaplasma but not *M. hominis* had significantly lower IgG ($p < 0.03$) and IgA ($p < 0.04$) (Kruskal-Wallis test) levels to *M. hominis* antigen. In addition, correlation coefficients between the two assays were low for IgG (<0.20) and IgA (<0.5), suggesting no significant cross-reactions. In the IgM class, a higher correlation (0.74) was observed. As would be predicted, the strongest correlation of IgM levels occurred in those individuals colonized by both *M. hominis* and *U. urealyticum* with no apparent cross-reactions in individuals with only one microorganism.

Finally, to determine if the presence of antibody to ureaplasma influenced detection of antibody titer changes; 12 acute and convalescent serum pairs were analyzed with the use of both the *M. hominis* and ureaplasma ELISAs. Six paired sera were from nongonococcal urethritis patients[8] and six were from patients with postpartum fever. Two individuals demonstrated changes in levels to both antigens; however, one decreased in titer to ureaplasma while increasing in titer to *M. hominis*. For the remaining individuals, all antibody changes were to a single microorganism even when specific antibody to the other microorganism was present.

Correlation of cultural isolation and ELISA. The agreement between cultural isolation and ELISA serology in 207 women is given in Table II. The best overall agreement was seen in the IgA class. In the IgG class, 48 of the 59 discrepancies (81%) were due to culturally negative, seropositive individuals. In the IgA class, however, 71% of the discrepancies were a result of culturally positive, seronegative individuals. Therefore, a positive serology in the absence of cultural isolation occurred most frequently in the IgG class ($p < 0.03$). A positive serology in the absence of cultural isolation rarely occurred in the IgA class ($p < 0.001$); further it was not uncommon to have cultural isolation in the absence of an IgA response.

Comparison of mycoplasmacidal and ELISA tests in postpartum fever patients. Twenty-seven individual sera (11 paired, one acute, and four convalescent) were evaluated. A serum sample was considered positive if the ELISA value was >2 SD above the normal serum value or if the mycoplasmacidal titer was ≥32. Eighteen and four sera were positive and negative, respectively, by both tests for an overall agreement of 81.5%. Four (14.8%) sera were positive by ELISA but negative by

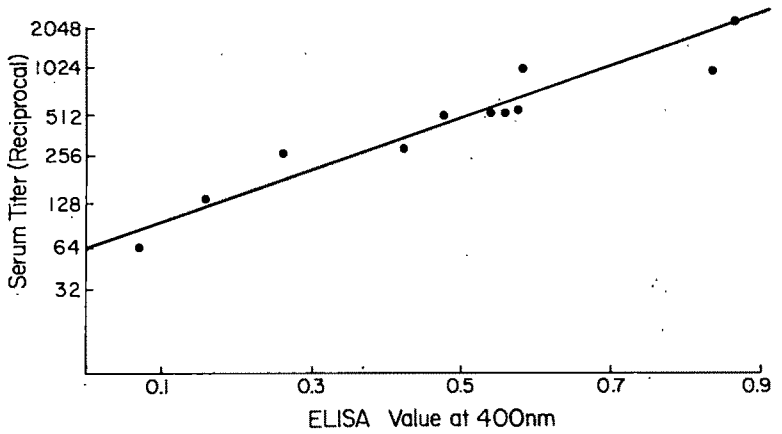


Fig. 5. Correlation of end point titer with ELISA A400 value for IgG. The reciprocal of the last dilution to give an A400 value ≥0.100 was deemed the end point titer. The end point titer for a serum was plotted versus the A400 value of the 1 : 128 dilution of that serum.

Table II. Agreement between cultural isolation of *Mycoplasma hominis* from the lower genital tract and presence of antibody in 207 women

Antibody class	*Culture positive (N = 45)*				*Culture negative (N = 162)*				*Total agreement*	
	Seropositive		*Seronegative*		*Seronegative*		*Seropositive*			
	n	%	*n*	%	*n*	%	*n*	%	*n*	%*
IgG†	34	76	11	24	114	70	48	30	148	71
IgM	29	64	14	31	140	86	22	14	169	82
IgA‡	25	56	20	44	154	95	8	5	179	86

*Includes both culture-positive, seropositive and culture-negative, seronegative individuals.
†Antibody class most likely to have seropositive but culture-negative individuals ($p < 0.03$).
‡Antibody class least likely to have seropositive but culture-negative individuals ($p < 0.001$).

mycoplasmacidal titer. Only one mycoplasmacidal-positive serum sample was not detected by ELISA.

Results obtained in the mycoplasmacidal test were dependent on the strain used as antigen whereas the results obtained by ELISA were relatively independent of the strain used as antigen (Table III). No single strain allowed detection of >40% of the positive sera when used in the mycoplasmacidal test. Although no statistically significant differences were found among *M. hominis* strains when used as ELISA antigens, strains PG21 and 4195 appeared to be the best antigens with 87% and 83% detection, respectively. For comparative purposes, *M. hominis* PG21 was chosen as the standard ELISA antigen and gave a high degree of correlation with results obtained for *M. hominis* strain W2 ($p < 0.04$) and all other strains tested ($p < 0.001$). All mycoplasmacidal-positive serum samples except 23 (Table III) could be identified with a combination of three strains (PG 21 and 4195 in combination with either 10 or W2).

Correlation of ELISA values and end point titers. In addition to the human immune and normal human serum reference serum standards, eight serum samples were selected at random and assayed to determine the relationship between ELISA optical density value and end point titer. The reciprocal of the lowest serum dilution giving an ELISA value ≥0.100 A400 unit was considered the end point titer. The optical density value obtained at the 1 : 128 dilution was chosen for the standard ELISA value. The regression line, slope 1.624, was obtained for IgG (Fig. 5). Regression lines obtained for IgM and IgA gave slopes of 3.416 and 5.628, respectively. On the basis of these regression lines, a difference in optical density values between acute and convalescent sera was deemed significant if it was ≥0.2 (IgG) or 0.1 (IgM, IgA).

Detection of class-specific antibody changes in postpartum fever patients. Acute and convalescent phase sera from 10 women with postpartum fever of mycoplasmal origin were tested for IgG, IgM, and IgA antibody. The differences between acute and convalescent levels are shown in Fig. 6. On the basis of the values established in the regression analysis, six of the 10 patients demonstrated a significant increase in one or

Table III. Comparison of *Mycoplasma hominis* strains as antigens in ELISA and mycoplasmacidal tests for detection of IgG in serum from women with postpartum fever of mycoplasmal origin

Serum	M. hominis *strains positive**	
	ELISA†	*Mycoplasmacidal test‡*
1	W2, 10, 93, PG21, 4195, 183	None
2	W2, 10, 93, 132, PG21, 4195, 183	4195
3	W2, 10, 93, 132, PG21, 4195, 183	4195, 183
4	W2, 10, 93, 132, PG21, 4195, 183	W2, 10, 93, 132, PG21, 4195
5	PG21	None
6	W2, 10, 93, 132, PG21, 4195, 183	W2, 93, PG21
7	W2, 10, 93, 132, PG21, 4195, 183	PG21
8	W2, 93, PG21, 4195, 183	PG21, 4195
9	W2, 10, 93, 132, PG21, 4195, 183	PG21, 4195, 183
10	10, 93, 132, PG21, 4195, 183	W2, 132, 183
11	W2, 10	10, 132, 4195, 183
12	W2, 10, 132, PG21, 4195, 183	None
13	4195	PG21
14	93, 132, PG21, 4195, 183	PG21
15	W2, 10, 93, 132, PG21, 4195, 183	PG21
16	W2, 10, 93, 132, PG21, 4195, 183	132, PG21, 183
17	W2, 10, 93, 132, PG21, 4195, 183	W2, 132, 183
18	PG21	None
19	W2, 10, 93, 132, PG21, 4195	PG21
20	W2, 10, 93, 132, PG21, 4195, 183	W2, 93, 132
21	W2, 10, 93, 132, PG21, 4195, 183	10, 132
22	W2, 10, 93, 132, PG21, 4195, 183	W2, 10, 93, 132
23	None	W2, 10, 93, 132, 4195

*For ELISA, antigens were cell lysates of the seven strains listed; for mycoplasmacidal tests, antigens were whole cells.

†A serum was ELISA positive if its value was greater than the normal serum value plus 2 SD.

‡A serum was mycoplasmacidal positive if its titer was ≥32. Strain 183 not tested in the mycoplasmacidal tests for Serum No. 4.

more antibody classes. Of these, one patient had a significant rise in all three antibody classes; four patients studied had concomitant rises in both IgG and IgM; and one had an increase in IgM. Of the remaining four patients, one had stable high levels of IgG and IgM; two had significant decreases in IgG and IgM, and the last had a significant decrease in IgG.

Comment

The antigenic determinant of *M. hominis* as well as the association of specific antigens with disease is relatively unknown.[11] Until the identification, purification, and characterization of these antigens with respect to their immunogenicity in humans is pursued, it is unlikely that a suitable purified antigen preparation will be available. When a mixed antigen preparation is used, primary considerations are the adsorption characteristics of the carrier surface and the competition among proteins for binding sites.[12] Serial dilutions of positive sera give shallow slopes with inadequate coating and adsorption of mixed antigen preparation.[12] With the *M. hominis* ELISA cell lysate antigen, the slopes observed for serial dilution of positive sera (Figs. 2 to 4) were steep whereas those of negative sera were shallow, indicating that use of a mixed antigen preparation was not a problem.[12]

The ubiquity of genital mycoplasmas coupled with the commensal nature of other mycoplasma species in the oral cavity of humans makes the determination of assay specificity difficult at best. Human sera with antibody to *M. pneumoniae* did not react with *M. hominis* antigen. No cross-reactions were observed between *M. hominis* and other mycoplasmas of human origin in the rabbit system.[11] However, a one-way cross-reaction between rabbit antisera to *U. urealyticum* and *M. hominis* antigens has been previously reported.[11] No significant correlation occurred between levels of antibody detected to *M. hominis* and *U. urealyticum* in 207 individuals. More important, individuals with significant changes in antibody levels to one antigen did not demonstrate changes to the other antigen, even in the antibody class (IgM) with the greatest degree of correlation. In a previous study,[8] individuals with antibody to one microorganism were seronegative to the other, thus suggesting that the antibody response is specific. The correlation of the *M. hominis* ELISA results with the mycoplasmacidal test as well as the ureaplasma ELISA with the metabolism inhibition test[8] is additional evidence for the validity of the assay. However, the potential for limited cross-reactions should always be considered, particularly with respect to IgM, in interpretation of data.

The observed discrepancies between cultural status and presence of antibody are predictable. A percentage

of culturally negative, serologically positive individuals is not uncommon[13]; whether this represents failure in culture technique, previous clearance of the organism, or nonspecificity is not known. Culturally negative individuals were most frequently positive in the IgG class, suggesting that these individuals may have had previous exposure to and subsequent clearance of *M. hominis*. It is of interest that 19 of the 48 IgG-positive, culturally negative individuals had evidence of tubal disease, suggesting that a previous infection with *M. hominis* may have occurred. The relatively low numbers of culturally negative individuals with specific IgA or IgM antibody (Table II) would also support a theory of clearance of the microorganism.

A significant association of antibody to *M. hominis* with infection has been shown in acute pyelonephritis,[3] postpartum fever,[7] and salpingitis.[14] In these instances, significant changes in titer correlated with both disease symptoms and cultural isolation. In the postpartum fever patients in the present study, significant changes were found in IgM (80%) and IgG (70%) levels, which is consistent with an active infection. In a recent study of acute pelvic inflammatory disease,[15] significant changes in antibody levels to *M. hominis* were observed in only 23% (IgG), 12% (IgA), and 10% (IgM) of patients. However, the cultural status of these individuals was not determined with respect to *M. hominis*, and other infectious agents including *Chlamydia trachomatis* and *Neisseria gonorhoeae* were isolated from 50% of the patients. It is possible that *M. hominis* was a causative agent in only a small percent of individuals with acute pelvic inflammatory disease, thus explaining the low incidence of significant changes in antibody levels, particularly in the IgM class.

The presence of IgM and IgA antibodies as well as IgG antibodies in healthy women (Table II) suggests that determination of class-specific antibody in a single serum sample may not necessarily be of diagnostic value. In addition, the seroconversion of women during normal pregnancies[4] suggests that interpretation of results obtained with acute and convalescent sera should be made in conjunction with clinical manifestations and cultural isolation. In a recent study of pregnant women,[16] the ELISA did not detect significant changes in antibody levels between acute and convalescent sera in asymptomatic patients but did detect significant changes in antibody levels in women with clinical amnionitis and in women in whom *M. hominis* was isolated from amniotic fluid.

Differences observed in the mycoplasmacidal test and ELISA may reflect either an increased sensitivity in the ELISA or a difference in the type of antibody detected. The mycoplasmacidal test detects only antibody that is biologically functional in the assay, whereas ELISA detects all class-specific antibody capable of forming immune complexes with the ELISA antigen. Failure of the ELISA to detect significant strain differences may be attributed to the type of antigen used. Soluble antigens of *M. hominis* are similar whereas differences exist in membrane protein antigens. The ELISA antigen contained both cytoplasmic and membrane antigens; thus dissimilarity due to the latter probably would not have been evident. Furthermore, strain specificity of the mycoplasmacidal test is affected by antigen concentration with loss of individual strain specificity at lower concentrations,[3] and the ELISA antigen contained only 10 μg of protein per milliliter.

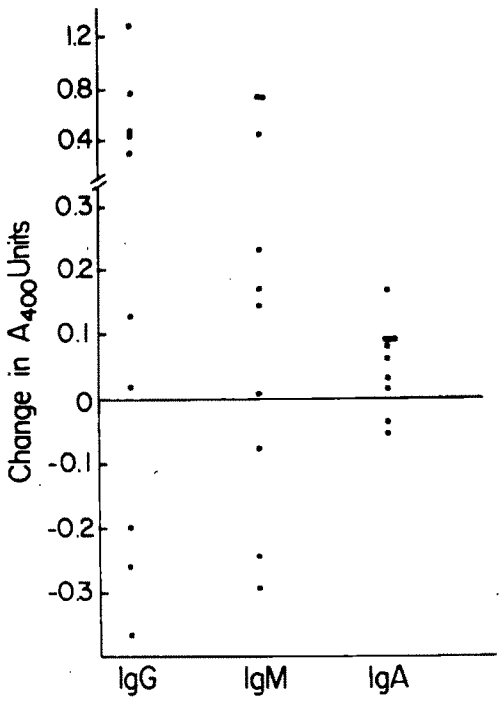


Fig. 6. Differences between acute and convalescent antibody in patients with postpartum fever. All differences represent convalescent value minus acute value for each patient.

The assays currently used to determine the presence of antibody require careful regulation of mycoplasma cell numbers and multiple strains of *M. hominis*. Few clinical laboratories possess the facilities or expertise to perform such assays. ELISA technology, however, has been readily adapted by commercial sources for routine use in the clinical setting. The ELISA presented in this study for detection of antibody to *M. hominis* is sensitive and correlates well with the mycoplasmacidal test. However, it is obvious that the ELISA, like other serologic assays, will require the use of antigen pools that reflect strain heterogeneity for optimal antibody detection. Our results suggest that type strain PG21 in combination with one or two other strains would be optimal for antibody detection. The optimal choice of strain combination may vary with the incidence of isolation within a given population, and this variable should be

considered when the assay is established. The sensitivity and simplicity of the ELISA in conjunction with its ability to detect class-specific antibody changes make this assay a potentially useful tool in both the clinical and research settings for evaluating the etiologic significance of *M. hominis* in disease.

We thank Dr. Edwin Bradley for help with statistical analysis of the data and Drs. J. G. Tully and J. W. Simecka for critical review of the manuscript.

REFERENCES

1. Taylor-Robinson D, McCormack W. The genital mycoplasmas. N Engl J Med 1980;302:1003, 1063.
2. Cassell GH, Davis RO, Waites KB, et al. Isolation of *Mycoplasma hominis* and *Ureaplasma urealyticum* from amniotic fluid at 16-20 weeks gestation: potential effect on outcome of pregnancy. Sex Transm Dis 1983;10:294.
3. Lin JS, Kass EH. Serological reactions of *Mycoplasma hominis:* differences among mycoplasmacidal, metabolic inhibition, and growth agglutination tests. Infect Immun 1974;10:535.
4. Lin JS, Radnay K, Kendrick MI, et al. Serologic studies of human genital mycoplasmas: distribution of titers of mycoplasmacidal antibody to *Ureaplasma urealyticum* and *Mycoplasma hominis* in pregnant women. J Infect Dis 1978;137:266.
5. Horowitz SA, Cassell GH. Detection of antibodies to *Mycoplasma pulmonis* by an enzyme-linked immunosorbent assay. Infect Immun 1978;22:161.
6. Wallace RJ, Alpert S, Brown K, et al. Isolation of *Mycoplasma hominis* from blood cultures in patients with postpartum fever. Obstet Gynecol 1978;51:181.
7. Platt R, Lin JS, Warren JW, et al. Infection with *Mycoplasma hominis* in postpartum fever. Lancet 1980;2:1217.
8. Brown MB, Cassell GH, Taylor-Robinson D, Shepard MC. Measurement of antibody to *Ureaplasma urealyticum* by an enzyme-linked immunosorbent assay and detection of antibody responses in patients with nongonococcal urethritis. J Clin Microbiol 1983;17:288.
9. Cassell GH, Younger JB, Brown MB, et al. Microbiologic study of infertile women at the time of diagnostic laparoscopy: association of *Ureaplasma urealyticum* with a defined subpopulation. N Engl J Med 1983;308:502.
10. Salonen EM, Vaheri A. Immobilization of viral and mycoplasmal antigens and of immunoglobulins on polystyrene surfaces for immunoassays. J Immunol Methods 1979;30:209.
11. Brown MB, Minion FC, Pritchard DG, et al. Antigens of *Mycoplasma hominis*. Sex Transm Dis 1983;10:247.
12. Kenny GE, Dunsmoor CL. Principles, problems, and strategies in the use of antigenic mixtures for the enzyme-linked immunosorbent assay. J Clin Microbiol 1983; 17:655.
13. Mardh PA, Westrom L. Antibodies to *Mycoplasma hominis* in patients with genital infections and in healthy controls. Br J Vener Dis 1970;46:390.
14. Mardh PA, Lind I, Svensson L, et al. Antibodies to *Chlamydia trachomatis, Mycoplasma hominis,* and *Neisseria gonorrhoeae* in sera from patients with acute salpingitis. Br J Vener Dis 1981;57:125.
15. Miettinen A, Paavonen J, Jansson E, et al. Enzyme immunoassay for serum antibody to *Mycoplasma hominis* in women with acute pelvic inflammatory disease. Sex Transm Dis 1983;10:289.
16. Gibbs RS, Cassell GH, Davis JK, St Clair PJ. Further studies on genital mycoplasmas in intra-amniotic infections: blood cultures and serologic response. Am J Obstet Gynecol 1986;154:717.

Fetal biophysical profile scoring: Selective use of the nonstress test

F. A. Manning, M.D., I. Morrison, M.B., I. R. Lange, M.B., C. R. Harman, M.D., and P. F. C. Chamberlain, M.D.

Winnipeg, Manitoba, Canada

The fetal biophysical profile score was modified by selective use of the nonstress test. In 2712 study patients (7851 tests) the incidence of nonstress test was reduced to 2.7% with no measurable effect or test accuracy. The nonstress test was most useful in evaluation of abnormal ultrasound monitored variables. (AM J OBSTET GYNECOL 1987;156:709-12.)

Key words: Fetus, biophysical profile score, dynamic ultrasound scan, nonstress test

The fetal biophysical profile score is reported as a reliable accurate method for antepartum fetal risk assessment.[1] This testing method is usually done in two sequenced steps: an initial dynamic ultrasound scan to record the presence or absence of fetal tone, gross body movements, and fetal breathing and a semiquantitative assessment of amniotic fluid volume followed by a nonstress test.[2] In a prospective blind clinical study in which perinatal morbidity and mortality were used as end points to judge specificity, sensitivity, predictive accuracy, measurement of fetal tone, gross body movement, fetal breathing movement, and amniotic fluid volume when compared to the nonstress test yielded superior results.[2] In a prospective randomized clinical trial comparing the nonstress test to the fetal biophysical profile score Platt et al.[3] reported a significant improvement in positive predictive value of abnormal outcome with fetal biophysical profile scoring and a better but nonsignificant improvement in negative predictive accuracy, sensitivity, and specificity.[3] The advantage, if any, of performing a nonstress test when the dynamic ultrasound-monitored variables of the fetal biophysical profile score are normal is unknown and forms the basis of this report. The purpose of this study was twofold: to determine the distribution of nonstress test results after initial dynamic ultrasound variable monitoring and to determine perinatal outcome, as reflected by perinatal mortality, achieved by primary dynamic ultrasound variable monitoring and selective use of the nonstress test.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Manitoba School of Medicine.

Received for publication June 3, 1986; revised August 11, 1986; accepted September 15, 1986.

Reprint requests: Dr. Frank A. Manning, Department of Obstetrics and Gynecology, WR-120, Women's Hospital, 735 Notre Dame Ave., Winnipeg, Manitoba, Canada R3E 0L8.

Table I. Study population by primary indication for referral

Indication for referral	*No. of patients*	*% of total*
Suspect intrauterine growth retardation	726	26.8
Hypertension	474	17.5
Diabetes		
Total	272	10
Gestational	164	6
Insulin-dependent	108	4
Decreased fetal movements	190	7
Large for dates	157	5.8
Antepartum hemorrhage	154	5.7
Spontaneous premature rupture of the membranes/premature labor	152	5.6
Suspect anomaly	111	4.1
Malpresentation (breech)	106	3.9
Maternal medical condition	91	3.4
Previous stillbirth	88	3.2
Rh isoimmunization	54	2
Twins	46	1.7
Other obstetrical indications	91	3.3
Total patients	2712	100
Total fetuses	2758	100

Method

The study population consisted of 2712 consecutive high-risk patients referred for antepartum fetal risk evaluation by fetal biophysical profile scoring; in total 2758 fetuses were studied (46 twin pregnancies). The indications for initial referrals are listed in Table I; the mean fetal age at referral was 36.4 ± 1.4 weeks (range, 26 to 44 weeks).

In each patient at each visit a dynamic ultrasound scan was done to record the presence or absence of fetal tone, gross body movements, and fetal breathing movements and to assess amniotic fluid volume; these variables were coded as normal or abnormal according to fixed criteria (Table II). If these initial variables were all normal, a nonstress test was not done unless specif-

Table II. Technique and interpretation of biophysical profile scoring

Biophysical variable	*Normal (score = 2)*	*Abnormal (score = 0)*
Fetal breathing movements	One or more episodes of ≥30 sec in 30 min	Absent or no episcde of ≥30 sec in 30 min
Gross body movements	Three or more discrete body/limb movements in 30 min (episodes of active continuous movement considered as single movement)	Two or less episodes of body/limb movements in 30 min
Fetal tone	One or more episodes of active extension with return to flexion of fetal limb(s) or trunk; opening and closing of hand considered normal tone	Either slow extension with return to partial flexion or movement of limb in full extension or absent fetal movement
Reactive fetal heart rate	Two or more episodes of acceleration of ≥15 bpm and of ≥15 sec associated with fetal movement in 20 min	Less than 2 episodes of acceleration of fetal heart rate or acceleration of <15 bpm in 40 min
Qualitative amniotic fluid	One or more pockets of fluid measuring ≥1 cm in two perpendicular planes	Either no pockets or a pocket <1 cm in two perpendicular planes

Table III. Distribution of test score results and comparison with nonstress test results

	Results		*Nonstress test*			
			Reactive		*Nonreactive*	
Ultrasound-monitored variable results	*No. of tests*	*% of total*	*No.*	*%*	*No.*	*%*
Four variables normal	7663	97.60	34	100	0	0
Three variables normal	153	1.94	92	64.4	51	35.6
Absent fetal breathing	123	–	80	65	43	35
Absent fetal movement	8	–	8	100	0	0
Absent tone	4	–	4	100	0	0
Decreased fluid	18	–	2	11.1	16	88.9
Two variables normal	16	0.22	2	12.5	14	87.5
Absent fetal breathing/tone	9	–	2	22	7	78
Absent tone/decreased fluid	7	–	0	0	7	100
One variable normal	15	0.19	0	0	15	100
Normal movement only	7	–	0	0	7	100
Normal fluid only	6	–	0	0	6	100
Normal fetal breathing only	2	–	0	0	2	100
All variables normal	4	0.05	0	0	4	100
Total	7851	–	128	60.4	84	39.6

ically requested. If one or more of the dynamic ultrasound variables were abnormal, a nonstress test was always done and interpreted according to fixed criteria (Table II). Repeated testing was recommended on a twice weekly basis in postdate pregnancies (>42 completed weeks) and diabetes (gestational and insulin-dependent) and on a weekly basis in all other high-risk groups. Criteria for intervention for test results were fixed. If the total score was 4 or less, intervention for fetal indication was always undertaken. A score of 6 was considered as indication for induction in the mature fetus (>36 weeks) and an indication for repeat testing in the immature fetus (<36 weeks); with a persistent score of 6 intervention was always undertaken. Oligohydramnios was always considered an indication for intervention regardless of the presence or absence of the other score variables, provided the membranes were intact and the fetal genitourinary system was judged as normal.

Perinatal mortality, both gross and corrected, was used to judge perinatal outcome and test accuracy. A corrected perinatal death was defined as death of a structurally normal nonisoimmunized fetus with a birth weight of at least 500 gm until and including the twenty-eighth day of neonatal life.

Results

A total of 7851 dynamic ultrasound tests were done in the 2712 study patients (mean, 2.9 tests per patient; range, one to 16 tests per patient). The four dynamic ultrasound-monitored variables were normal in 7663 of 7851 tests (97.60%). In 153 tests (1.94%) a single ultrasound variable was abnormal, in 16 tests two variables were abnormal (0.22%), in 15 tests three variables were abnormal (0.19%), and in four tests all variables were abnormal (0.05%). The frequency of abnormality of each variable is listed in Table III. In decending order of frequency abnormal tests were absent fetal breathing (149 tests), absent fetal tone (39 tests), abnormal amniotic fluid volume (38 tests), and abnormal fetal movements (20 tests).

A nonstress test was performed as an additional test

in 212 of the 7851 tests (2.7%). In 34 instances a nonstress test was done by physician request despite all four ultrasound-monitored variables being normal; all 34 tests were reactive (100%). In 178 instances a nonstress test was done because one or more ultrasound-monitored variables were abnormal (2.26% of total tests). The incidence of an abnormal nonstress test result increased progressively as the number of abnormal ultrasound-monitored variables increased, ranging from 35.6% nonreactive tests with one abnormal ultrasound variable to 100% nonreactive tests when three or more ultrasound-monitored variables were abnormal (Fig. 1).

Seventeen perinatal deaths occurred among the 2752 fetuses studied (gross perinatal mortality, 6.17 per 1000), of which 10 were stillbirths (gross stillbirth rate, 3.63 per 1000). Thirteen of the 17 perinatal deaths were due to either major structural anomaly (10 deaths) or severe isoimmunization and fetal hydrops (three deaths). The corrected perinatal mortality in this study population was 1.45 per 1000 (four deaths). In two of these corrected perinatal deaths fetal death occurred within 1 week of a normal test result, yielding a false negative rate of 0.73 per 1000; in one instance the death resulted from massive abruptio placentae and in the other the cause of death was undetermined and presumed asphyxial in origin. The remaining two corrected deaths occurred despite intervention for an abnormal score and are also presumed to be asphyxial in origin.

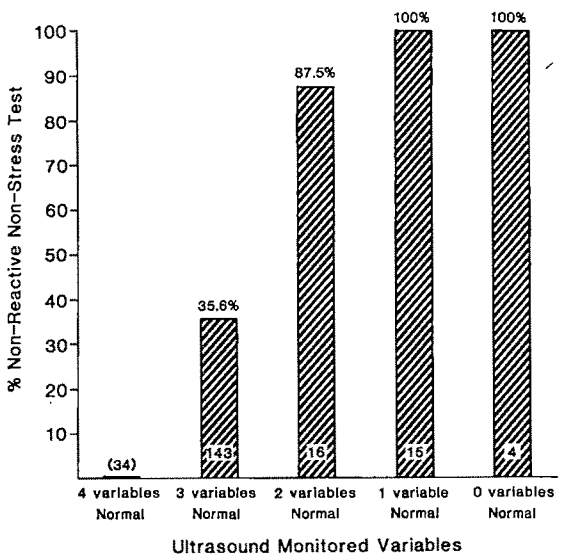


Fig. 1. Histogram of distribution of nonreactive nonstress test results as compared to the results of the dynamic ultrasound-monitored variables. A detailed breakdown of individual variable results to the nonstress test results is given in Table III.

Comment

Before accepting a change in a testing method it is essential to demonstrate that the proposed modification yields similar or superior test accuracy and is of some clinical advantage. The modification of the fetal biophysical profile score by selective use of the nonstress test component as described here appears to satisfy these criteria. The modified testing method yielded a gross perinatal mortality of 6.17 per 1000, a corrected perinatal mortality of 1.43 per 1000, and a false negative rate of 0.73 per 1000. In a large prospective clinical study using the complete fetal biophysical score, the comparable rates were a gross perinatal mortality of 7.37 per 1000, a corrected perinatal mortality of 1.90 per 1000, and a false negative rate of 0.65 per 1000.[1] Other studies using the complete fetal biophysical profile score have reported similar results.[4-6] Therefore the modification studied, that is, selective use of the nonstress test, has not produced any measurable decrease in test accuracy.

The main clinical advantage of this modification is the elimination of the need for performing the nonstress test in all but 2.7% of patients, thereby reducing testing time and fetal exposure to additional Doppler ultrasound energy. The time required to perform the dynamic ultrasound monitoring in this study was not recorded, but in previous studies it is reported that in 97% of instances these variables when normal will be observed within the first 10 minutes.[1] Since in this study, as in previous studies, the large majority of dynamic ultrasound tests will yield normal variables (97.73%), testing time is short and patients may be assessed more rapidly. In this center the modification has dramatically improved the efficacy of the testing unit, shortened the duration of the patient's visit, and increased the number of tests that may be performed daily. This advance is achieved without any measurable change in predictive accuracy.

The relationship between the ultrasound-monitored variables and the nonstress test results is intriguing. The acute fetal biophysical variables measured, fetal breathing, tone, movement, and heart rate acceleration with movement, are known to be influenced by fetal sleep-wake cycles.[7] In the small number of cases studied when all ultrasound-monitored variables were normal, the nonstress test was always reactive (34 cases); whether this association is invariable cannot be determined from this sample size. This association suggests that when testing occurs during a time when the normal fetus is in active sleep, all acute variables are likely to be normal. In contrast, when any single ultrasound-monitored variable other than amniotic fluid volume, is abnormal, the addition of the nonstress test is useful in differentiating the fetus in quiet sleep from the compromised fetus. In this study absent fetal breathing movements were the most common single abnormal variable. Fetal breathing movements are known to be periodic in nature and markedly influenced by sleep state.[8] When fetal breathing movements were absent

for 30 minutes, an immediate nonstress test yielded normal results in 65% of cases (80 of 123 tests). This relatively high frequency of a reactive nonstress test in such cases is probably the result of the extended testing time with spontaneous fetal transition from quiet to active sleep. Since the predictive accuracy of the nonstress test and fetal breathing movements when considered as single variables is equal, the presence of a reactive nonstress test despite absent fetal breathing movements may be considered reassuring.[9] A similar effect of sleep state may explain the high probability of a reactive nonstress test despite either abnormal fetal movements or tone and again is considered reassuring. Decreased amniotic fluid volume shows a distinctly different association with the nonstress test results, in this small sample size (n = 38) yielding a nonreactive test in 33 instances (86.9%). Since decreased amniotic fluid volume is not acutely influenced by fetal sleep state and is associated with a sharp increase in perinatal compromise and mortality,[10, 11] this high correlation is not unexpected. When two or more ultrasound-monitored variables were abnormal, the probability of a nonreactive nonstress test result rose dramatically. Since the risk of perinatal compromise rises as more ultrasound-monitored variables become abnormal,[2] this association is more likely due to intrauterine asphyxia and less likely due to influence of fetal sleep state.

In theory the fetal biophysical profile score measures immediate fetal condition as reflected by the presence of central nervous system–regulated acute biophysical variables (movement, breathing, tone, and heart rate reactivity) and chronic fetal condition as reflected by amniotic fluid volume. As demonstrated in this study and others,[2, 4-6] the predictive value of these acute variables are interchangeable and no advantage is gained provided all or most are normal. In contrast, amniotic fluid volume determination is a separate and critical variable improving predictive accuracy in both the general high-risk groups and specific high-risk subsets such as the postmature fetus[12] and the fetus with intrauterine growth retardation.[13] We are of the opinion then that any method of risk assessment using acute variables, be they the nonstress test alone or dynamic ultrasound-monitored variables such as fetal breathing, movement, and tone, also requires consideration of amniotic fluid volume to achieve maximal predictive accuracy. If this principle were accepted, then at least two testing strategies would arise. One approach would be to record the nonstress test and then use B-mode ultrasound to assess amniotic fluid volume. To date the results of clinical testing of this method are not reported. This present study presents the alternative approach, that is, combining simultaneous amniotic fluid volume and fetal breathing, movement, and tone as the primary test, reserving the nonstress test for cases in which one or more of these variables are abnormal. This approach yielded improvement in testing logistics without compromising accuracy. Whether this approach is better than the alternate nonstress test and amniotic fluid volume determination cannot be determined but warrants further investigation.

REFERENCES

1. Manning FA, Morrison I, Lange IR, Harman CR. Fetal assessment based on fetal biophysical profile scoring: experience in 12,620 referred high-risk pregnancies. I. Perinatal mortality by frequency and etiology. Am J Obstet Gynecol 1985;151:343-50.
2. Manning FA, Platt LD, Sipos L. Antepartum fetal evaluation: Development of a fetal biophysical profile score. Am J Obstet Gynecol 1980;136:787-95.
3. Platt LD, Walla C, Paul RH, et al. A prospective trial of the fetal biophysical profile score vs. the nonstress test in the management of high risk patients. Am J Obstet Gynecol 1985;153:624-33.
4. Baskett TF, Gray JH, Prewett ST, et al. Antepartum assessment using a fetal biophysical profile score. Am J Obstet Gynecol 1983;148:630-3.
5. Platt LD, Eglington GS, Sipos L. Further experience with the Fetal Biophysical Profile Score. Obstet Gynecol 1983; 61:480-5.
6. Vintzileos AM, Campbell WA, Ingardia PT, et al. The fetal biophysical profile score and its predictive value. Obstet Gynecol 1983;62:271-8.
7. Natale R, Nasello-Paterson C. The effect of electrical stimulation on behavioural states in the fetal lamb. Am J Obstet Gynecol 1986;154:321-8.
8. Boddy K, Dawes GS. Fetal breathing. Br Med Bull 1976;31:3-7.
9. Manning FA, Platt LD, Sipos L, et al. Fetal breathing movements and the nonstress test in high-risk pregnancies. Am J Obstet Gynecol 1979;135:511-5.
10. Chamberlain PFC, Manning FA, Morrison I, et al. Ultrasound evaluation of amniotic fluid volume. I. The relationship of marginal and decreased amniotic fluid volumes to perinatal outcome. Am J Obstet Gynecol 1984;150:245-9.
11. Bastide A, Manning FA, Harman CR, et al. Ultrasound evaluation of amniotic fluid: outcome of pregnancies with severe oligohydramnios. Am J Obstet Gynecol 1986; 154:895-900.
12. Johnson JM, Harman CR, Lange IR, et al. Biophysical profile scoring in the mangement of the postterm pregnancy: an analysis of 307 patients. Am J Obstet Gynecol 1986;154:269-73.
13. Manning FA, Hill LD, Platt LD. Qualitative amniotic fluid volume determination by ultrasound: antepartum detection of intrauterine growth retardation. Am J Obstet Gynecol 1981;139:254-8.

Embryonic trunk circumference: A new biometric parameter for estimation of gestational age

E. Albert Reece, M.D., Angela L. Scioscia, M.D., Jacqueline Green, M.S., R.D.M.S., Theresa Z. O'Connor, M.P.H., and John C. Hobbins, M.D.

New Haven, Connecticut

One hundred thirty-one uncomplicated, singleton pregnancies between 7 and 12 weeks of gestation were ultrasonically examined. Evaluation included confirmation of cardiac activity and measurements of fetal crown-rump length and trunk circumference. Both crown-rump length and trunk circumference were curvilinearly correlated with gestational age. A second- and third-degree polynomial equation best described this curvilinear relationship between trunk circumference and gestational age and between crown-rump length and gestational age. The crown-rump length and trunk circumference were similar in their prediction of gestational age, but the combination of the two parameters did not improve the prediction of gestational age. A nomogram of gestational age as predicted by trunk circumference was generated. These data provide an additional measurement for the estimation of gestational age in the first trimester of pregnancy. (AM J OBSTET GYNECOL 1987;156:713-5.)

Key words: Ultrasound, abdominal circumference, embryonic age, gestational age

In 1973 Robinson[1] described the ultrasonic measurement of the fetal crown-rump length and its correlation with gestational age. Subsequent reports have shown that a crown-rump measurement during the first trimester is predictive of gestational age within 4.7 days. The original study was performed on B-mode scanners and was subsequently confirmed with real-time sonography.[2-5] When measured properly the crown-rump length can estimate the gestational age with a high degree of accuracy. However, it is the only measurement with which to estimate gestational age in the first trimester. Additional measurements for the corroboration of age are currently unavailable.

The purpose of this study was to determine whether an additional biometric parameter could be generated for estimating the embryonic age that would serve as a useful adjunct in corroborating or rejecting the prediction of age obtained by the crown-rump length.

Patients and methods

Patients with uncomplicated pregnancies were recruited from the private and university practices for ultrasound examinations. Patients were included in this study if they had regular menses, if the date of the last menstrual period was known, and if the gestational age was confirmed by embryonic crown-rump length measurement (within 4 days of each other). All pregnancies were singleton gestations. The examinations were conducted with a 5.0 MHz sector scanner or Technicare Aloka 280 SL (General Electric RT 3000, Milwaukee, Wisconsin) with dynamic focusing capability in which optimal resolution was possible at varying depths of tissue penetration. Each examination included a measurement of crown-rump length and trunk circumference. The crown-rump length was measured in the longest axis of the fetus, excluding limbs, in the manner described by Robinson and Fleming.[1, 5] The trunk circumference was measured perpendicular to the long axis at a point just caudal to the cardiac pulsation (Fig. 1). The trunk circumference was calculated as the mean of two trunk diameters multiplied by 3.14. The data, which comprised a single examination from each patient, were analyzed by producing scatter plots, then determining the polynomial equation that best described the relationship between crown-rump length and trunk circumference and gestational age as calculated by the last menstrual period. In addition, a multiple regression analysis was used to determine whether the combination of trunk circumference and crown-rump length would predict gestational age more precisely than crown-rump length alone.

Results

One hundred thirty-one uncomplicated pregnancies between 7 and 12 weeks of gestation were examined, with low interobserver error. The trunk circumference

From the Division of Maternal-Fetal Medicine of the Department of Obstetrics and Gynecology and the Biostatistics Section of the Department of Epidemiology and Public Health, Yale University School of Medicine.

Received for publication August 26, 1986; accepted September 24, 1986.

Reprint requests: Dr. E. Albert Reece, Department of Obstetrics and Gynecology, Yale University School of Medicine, 333 Cedar St., P. O. Box 3333, New Haven, CT 06510-8063.

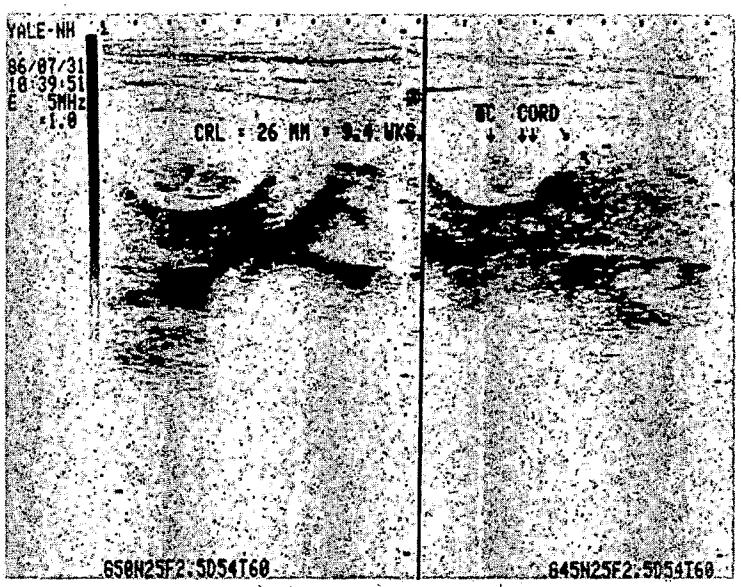


Fig. 1. Ultrasound picture of a first-trimester conceptus, with the embryo seen in longitudinal view and its crown-rump length *(CRL)* measured. Note also the cross-sectional *(TC)* view of the embryo taken below the cardiac pulsation. The umbilical cord *(CORD)* can be seen (⇊) as it enters the fetal abdomen and also as it inserts itself into the placenta (⇵).

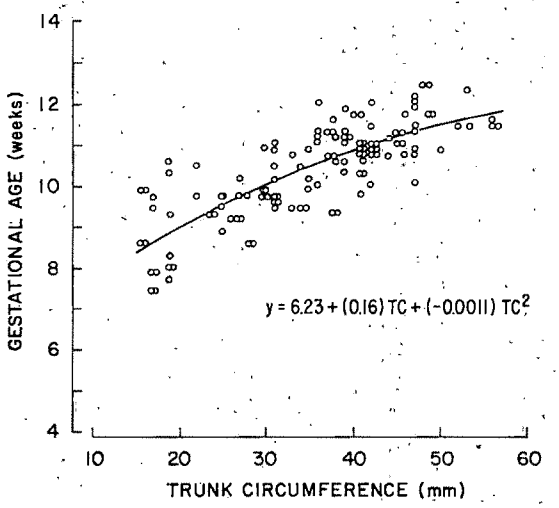


Fig. 2. Scattergram of trunk circumference measurements (X axis) predicting gestational age (Y axis), with fitted second-degree polynomial regression line.

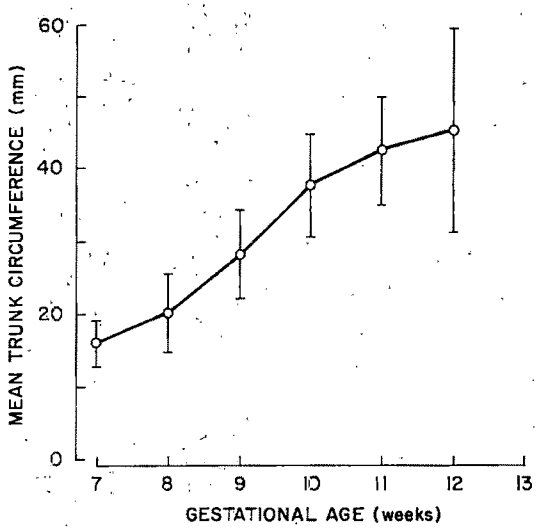


Fig. 3. Mean trunk circumference measurements ± 2 standard deviations plotted against gestational age.

was highly correlated with gestational age and with the crown-rump length ($p < 0.0001$). The growth of trunk circumference over time as a measure of gestational age was curvilinear and was best represented by a second-degree polynomial equation (Figs. 2 and 3). Growth of the crown-rump length with age and with trunk circumference was also curvilinear and best described by a third-degree polynomial equation (Fig. 4). Tests of higher degree terms showed them to be nonsignificant. The equations describing the above relationships are:

$$GA = 6.23 + (0.16)TC + (-0.0011)TC^2;\ R^2 = 0.58$$

$$GA = 5.23 + (2.53)CRL + (-0.345)CRL^2 + (0.0172)CRL^3;\ R^2 = 0.71$$

A nomogram of gestational age as predicted by the trunk circumference was generated (Table I). Multiple regression analysis revealed that the combination of trunk circumference and crown-rump length did not improve the prediction of gestational age over that using crown-rump length alone.

Table I. Embryonic trunk circumference and estimation of gestational age

Trunk circumference (mm)	*Gestational age ± 2 SD* (wk)*
10	7.72 ± 0.5
12	7.99 ± 0.4
15	8.39 ± 0.3
17	8.64 ± 0.3
20	8.99 ± 0.2
22	9.22 ± 0.2
25	9.55 ± 0.2
27	9.75 ± 0.2
30	10.05 ± 0.2
32	10.23 ± 0.2
35	10.49 ± 0.2
37	10.65 ± 0.2
40	10.88 ± 0.2
42	11.02 ± 0.2
45	11.22 ± 0.2
47	11.33 ± 0.2
50	11.50 ± 0.2
55	11.72 ± 0.3
60	11.89 ± 0.5
65	12.01 ± 0.7
70	12.07 ± 0.9

*Overall, 2 SD = ±3 days.

Comment

Embryonic growth observed by measuring the long axis of the fetus or the crown-rump length follows a curvilinear growth pattern over time and predicts gestational age in the first trimester of pregnancy with an accuracy of ±4.7 days.[2] Chervenak et al.,[6] using basal body temperature to date conception, have shown the crown-rump length to be a more accurate measure of gestational age than the human chorionic gonadotropin titer done during the first trimester. Until now, the crown-rump length measurement has been the most accessible and accurate predictor of gestational age and the only available method for estimating gestational age in the first trimester. The current study has demonstrated that the growth of the embryonic trunk circumference is similar to that of the crown-rump length and provides an additional means of estimating gestational age in the first trimester with an accuracy of ±3 days.

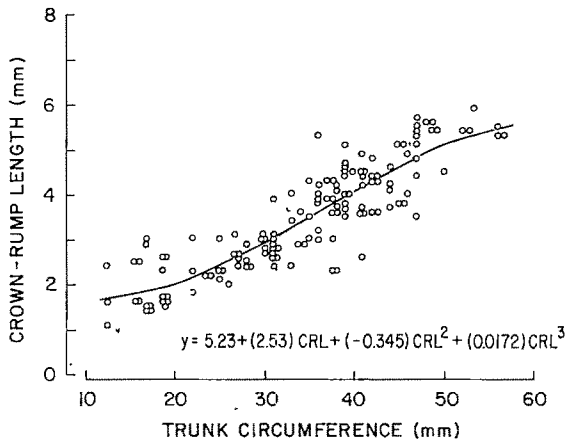


Fig. 4. Scattergram of crown-rump length plotted against trunk circumference, with fitted third-degree polynomial regression line.

The adjunctive utilization of trunk circumference measurements to estimate age may serve to confirm or reject information derived from crown-rump length determinations.

REFERENCES

1. Robinson HP. Sonar measurements of fetal crown-rump length as means of assessing maturity in first trimester pregnancy. Br Med J 1973;4:28-31.
2. Nelson LH. Comparison of methods for determining crown-rump measurement by real-time ultrasound. JCU 1981;9:67-70.
3. Bovicelli L, Orsini LF, Rizzo N, Calderoni I. Pazzaglia FL, Michelacci L. Estimation of gestational age during the first trimester of real-time measurement of fetal crown-rump length and biparietal diameter. JCU 1981;9:71-5.
4. Sande HA, Reiersten O. Crown-rump length: a comparison of real-time scanning and conventional compound scanning. The inter-individual measuring variation between two operators. Ultrasound Med Biol 1979;5:279-81.
5. Robinson HP, Fleming JE. A critical evaluation of sonar "crown-rump length" measurements. Br J Obstet Gynaecol 1975;82:702-10.
6. Chervenak FL, Brightman RC, Thornton J, Berkowitz GS. An analysis of sonographically determined fetal-crown rump length and natural serum human chorionic gonadotropin as predictors of gestational age [Abstract]. In: Scientific Abstracts of the thirty-third annual meeting of the Society for Gynecologic Investigation, Toronto, Ontario, Canada, March 26-29, 1986.

Levels of epidermal growth factor binding in third-trimester and term human placentas: Elevated binding in term placentas of male fetuses

Melinda J. Brown, Ph.D., Christine L. Cook, M.D., John L. Henry, M.S., and Gregory S. Schultz, Ph.D.
Louisville, Kentucky

Specific binding of iodine 125–labeled epidermal growth factor was measured in membrane homogenates of third-trimester and term human placentas of male and female fetuses. All placentas ($n = 35$) generated curvilinear Scatchard plots, and all placentas had similar equilibrium dissociation constants for both high-affinity (210 pmol/L) and low-affinity (830 pmol/L) binding sites. Mean maximum number of available binding sites of term placentas from male fetuses (330 ± 110 fmol/mg protein; $n = 11$) was significantly higher ($p < 0.001$) than that of female fetuses (170 ± 80 fmol/mg protein, $n = 13$). Occupation of epidermal growth factor receptors by endogenous epidermal growth factor could not account for the difference in levels of epidermal growth factor binding. Kendall's rank order correlation test demonstrated a significant positive correlation of the gestational age in weeks with the maximum number of epidermal growth factor receptors in placentas from male fetuses ($p < 0.002$; $T = 0.527$; $n = 17$) and female fetuses ($p < 0.01$; $T = 0.404$; $n = 18$). These results indicate (1) that there is a sexual dimorphism in the level of epidermal growth factor receptors in placentas of male and female fetuses during the third trimester and (2) that the level of epidermal growth factor receptors increases during the third trimester for placentas of both male and female fetuses. (Am J Obstet Gynecol 1987;156:716-20.)

Key words: Epidermal growth factor binding, placenta, development

The presence of high levels of epidermal growth factor binding in placental tissue[1] strongly suggests that epidermal growth factor plays important physiologic roles in fetoplacental function. For example, epidermal growth factor has been shown to stimulate the secretion of human chorionic gonadotropin and human placental lactogen in cultures of normal term human placental cells[2] and to stimulate the secretion of human chorionic gonadotropin[3, 4] and progesterone[5] in cultured human choriocarcinoma cells. All of these biologic actions of epidermal growth factor are presumably mediated by receptors for epidermal growth factor, and thus the response of tissues to epidermal growth factor may be regulated in part by their levels of epidermal growth factor receptor. Regulation of epidermal growth factor receptor levels in the placenta is not well understood. We have measured levels of epidermal growth factor binding in placental membranes to determine whether receptor levels change during development in the third trimester and whether differences exist between male and female placental tissue at term.

From the Departments of Biochemistry and Obstetrics and Gynecology, University of Louisville School of Medicine.
Received for publication August 25, 1986; accepted September 15, 1986.
Reprint requests: Dr. Gregory Schultz, Department of Biochemistry, University of Louisville, Louisville, KY 40292.

Material and methods

Common chemicals, chloramine T, and testosterone, were obtained from Sigma Chemical Co., St. Louis, Missouri. Sodium iodide I-125 solution was obtained from Amersham Corp., Arlington Heights, Illinois. Gelman filters were obtained from Gelman Instruments, Quincy, Massachusetts.

Tissue collection and preparation. Preterm and term human placentas were obtained after normal vaginal delivery or after cesarean section. All placentas were immediately placed on ice, brought to the laboratory, and frozen. Placentas were homogenized with a Polytron homogenizer (Brinkman Instruments, Westbury, New Jersey) at 4° C in buffer (10 mmol/L Tris hydrochloride [pH 7.0], 250 mmol/L sucrose, and 1 mmol/L calcium chloride), then filtered through cheesecloth as previously described.[6] Homogenates were stored in aliquots at −20° C until assayed for binding. Protein content in each homogenate was determined after membranes were digested at 80° C in 0.1 mol/L sodium hydroxide containing 0.1% sodium dodecyl sulfate by the method of Lowry et al.,[7] with bovine serum albumin used as the standard.

Isolation and iodination of mouse epidermal growth factor. Mouse epidermal growth factor was isolated and purified to homogeneity from the submandibular glands of testosterone-stimulated male mice according

to the procedure of Savage and Cohen.[8] Mouse epidermal growth factor was iodinated with chloramine T, and concentrations of mouse epidermal growth factor solutions were determined by spectrophotometric absorption at 280 nm (1 mg/ml = 3.09 absorption units).

Epidermal growth factor binding studies. Specific epidermal growth factor binding was measured as described previously.[6] Briefly, in experiment 1, aliquots (100 μl) of homogenate containing 50 μg of protein were incubated for 2 hours at 22° C with increasing concentrations of iodine 125–labeled mouse epidermal growth factor (9 to 5100 pmol/L; specific activity, 226 μCi/μg) in the presence and absence of 0.2 μmol/L unlabeled epidermal growth factor. Total volume of the incubation mixture was brought to 200 μl by addition of buffer (150 mmol/L sodium chloride and 1.0% bovine serum albumin). After incubation was complete suspensions were collected by filtration on 0.2 μm Gelman filters (GA-8), washed with 10 ml of ice-cold buffer (10 mmol/L Tris hydrochloride, pH 7.0), and counted in a Searle Model 1119 autocounter (Searle Laboratories, Chicago, Illinois) with a counting efficiency of 63% for ^{125}I. Alternatively, in experiment 2, after the material was incubated as above with increasing concentrations of ^{125}I-labeled epidermal growth factor (5 to 5000 pmol/L; specific activity, 343 μCi/μg), 3 ml of cold buffer was added to membrane suspensions, and suspensions were then centrifuged at 10,000 × g for 20 minutes at 4° C. Supernatant solutions were poured off, and pellets were counted in a Beckman 4000 Biogamma counter (Beckman Instruments Inc., Fullerton, California) with an efficiency of 70% for ^{125}I. We have demonstrated previously that binding of ^{125}I-labeled epidermal growth factor to human placental membrane reaches equilibrium after 2 hours at 22° C and that binding of ^{125}I-labeled epidermal growth factor is highly specific with these techniques.[9]

Specific binding data were calculated by subtracting the amount of epidermal growth factor bound in the presence of unlabeled mouse epidermal growth factor from that bound in the absence of unlabeled mouse epidermal growth factor. Specific binding was transformed by the method of Scatchard, and equilibrium dissociation constants for high- and low-affinity sites and maximum number of available binding sites were determined by use of the ISIS-12 computer program (National Institutes of Health, Bethesda, Maryland).[10] This program estimated affinities and binding capacities of two heterogeneous binding sites by the limiting slope technique. Identity of the sex of the fetus was masked until data for placental homogenates were calculated.

Rapid debinding of endogenous epidermal growth factor and rebinding of ^{125}I-labeled epidermal growth factor. Incubation of ^{125}I-labeled epidermal growth factor with placental membrane homogenates under the conditions described above measures unoccupied epidermal growth factor receptors. To measure the percent of epidermal growth factor receptors occupied by endogenous epidermal growth factor, membranes were stripped of bound epidermal growth factor by brief exposure to low pH at cold temperature as described previously.[11] Specific epidermal growth factor binding was then measured and compared with the level of epidermal growth factor binding in companion membranes not exposed to debinding conditions. Briefly, membranes were exposed to 40 mmol/L sodium acetate, pH 4.5, at 4° C for 4 minutes, then centrifuged at 10,000 × g for 2 minutes at 4° C with a Beckman microfuge. The supernatant solution was removed, and membranes were immediately resuspended in 40 mmol/L sodium acetate, pH 7.0. Specific binding was then measured as described above. This procedure removed >90% of ^{125}I-labeled epidermal growth factor bound to hepatocytes in culture membranes, with <10% loss of epidermal growth factor rebinding capacity in vitro.[11]

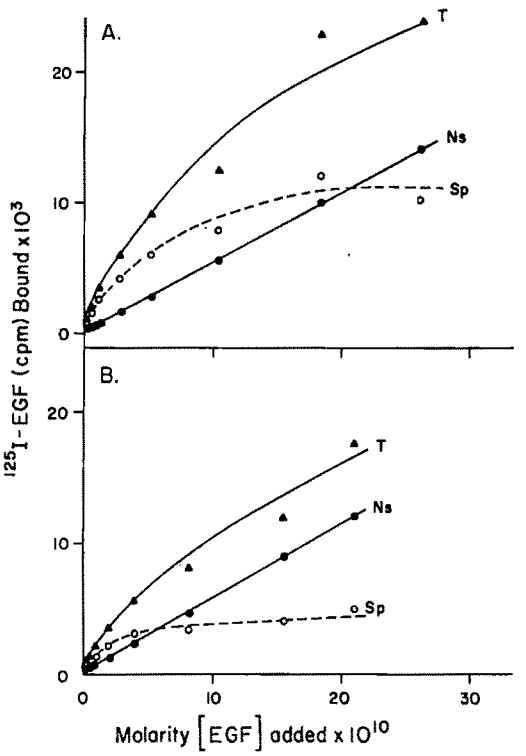


Fig. 1. Titration of ^{125}I-labeled epidermal growth factor binding to homogenates of normal term placentas. Aliquots of placental homogenate containing 50 μg of protein were incubated with increasing concentrations of ^{125}I-labeled mouse epidermal growth factor in the presence or absence of 0.2 μmol/L unlabeled epidermal growth factor for 2 hours at 22° C. Specific binding (○) was calculated by subtracting nonspecific binding (●) from total binding (▲). *A*, Epidermal growth factor binding to placenta of male fetus. *B*, Epidermal growth factor binding to placenta of female fetus.

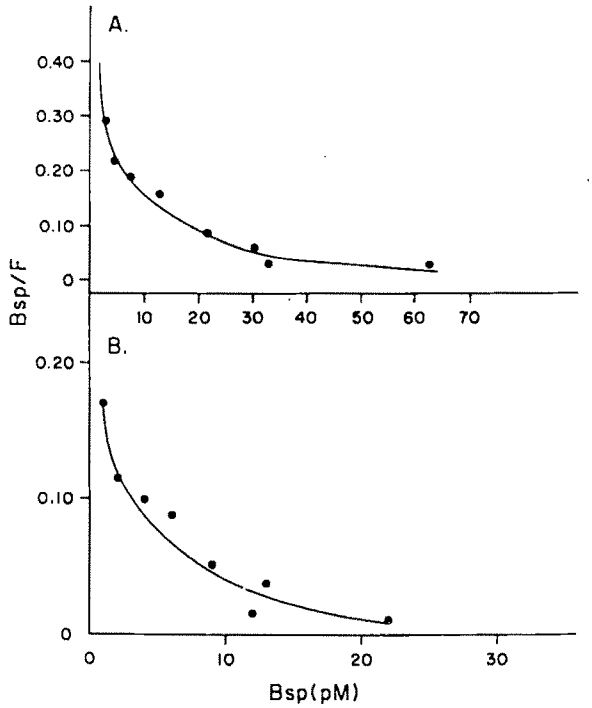


Fig. 2. Scatchard plots of specific ^{125}I-labeled epidermal growth factor binding from Fig. 1. Equilibrium dissociation constants and the maximum number of available binding sites were determined by the ISIS-12 program. *A*, Term placenta of male fetus. *B*, Term placenta of female fetus.

Statistical analysis. Kendall's rank order correlation test and Student's *t* test were used for statistical analysis of the data.

Results

Specific binding to term placentas. Representative titration curves of specific binding of ^{125}I-labeled epidermal growth factor to crude membranes from term placentas of male and female fetuses are shown in Fig. 1. Nonspecific binding was linear for placentas from both male and female fetuses (mean $R^2 = 0.99$; $n = 24$) over the range of ^{125}I-labeled epidermal growth factor added and represented approximately 60% of total binding at high levels of ^{125}I-labeled epidermal growth factor. Specific binding approached a plateau at approximately 1 nmol/L, indicating saturation of specific binding sites in homogenates of 20 of 25 term placentas. The five placental homogenates not approaching saturation were all from male fetuses. The average half-saturation concentration of ^{125}I-labeled epidermal growth factor estimated from titration curves was 710 ± 90 pmol/L and agreed well with the average low-affinity dissociation constant calculated from Scatchard plots.

Scatchard transformation of specific binding generated curvilinear plots in all term placentas (Fig. 2). Similar equilibrium dissociation constants were derived in all male (n = 11) and all female placentas (n = 13) for low-affinity binding sites (dissociation constant = 720 ± 180 pmol/L) and for high-affinity binding sites (dissociation constant = 140 ± 60 pmol/L).

The average of the maximum available binding sites extrapolated from Scatchard plots from experiment 1 of term placentas from six male fetuses was significantly higher ($p < 0.005$ by *t* test) than the average of the maximum binding sites of term placentas from eight female fetuses (β_{max} = 310 ± 40 and 130 ± 20 fmol/mg protein, respectively). In experiment 2, additional placentas from five male and five female fetuses were assayed for specific epidermal growth factor binding. As found in experiment 1, Scatchard transformation of specific epidermal growth factor binding indicated that placental membranes from male fetuses bound significantly ($p < 0.05$ by *t* test) more epidermal growth factor than placental membranes from female fetuses (360 ± 90 fmol/mg of protein for male and 230 ± 70 fmol/mg of protein for female fetuses). When data from the two experiments are combined, placentas from 11 male fetuses bound significantly ($p < 0.001$ by *t* test) more epidermal growth factor than placentas from 13 female fetuses (330 ± 100 versus 170 ± 80 fmol/mg of protein).

It is interesting to note that when specific epidermal growth factor binding was calculated by use of values obtained at a near-saturating concentration (1 nmol/L) of ^{125}I-labeled epidermal growth factor from data collected in experiment 2, the average level of binding in male fetuses (100 ± 40 fmol/mg of protein) was higher than the average level of binding in female fetuses (90 ± 30 fmol/mg of protein); however, the difference did not reach statistical significance ($p < 0.5$). Thus it is important to analyze placental binding by conducting multipoint titration analysis with high levels of epidermal growth factor and to analyze specific binding by Scatchard transformation.

When placental membranes from four male and four female term fetuses were incubated with ^{125}I-labeled epidermal growth factor and washed by centrifugation as described in experiment 2, then subjected to rapid debinding conditions, 85% ± 5% of the specifically bound ^{125}I-labeled epidermal growth factor was removed. Thus endogenous epidermal growth factor bound to receptors of male or female fetuses also should be removed equally well by the rapid debinding conditions. To determine the extent of occupancy of epidermal growth factor receptors by endogenous epidermal growth factor, placental membranes from four male and four female term fetuses were subjected to rapid debinding conditions, assayed for specific epidermal growth factor binding, and then compared with companion samples of placental membranes not subjected to rapid debinding conditions. Recovery of spe-

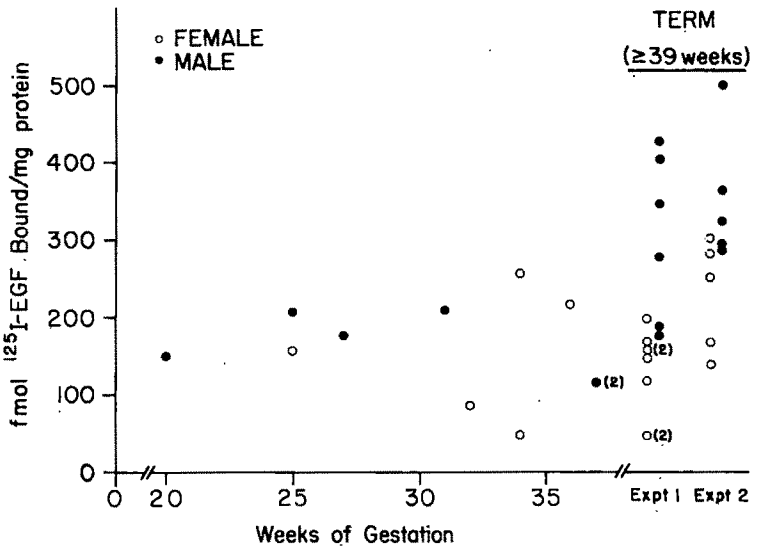


Fig. 3. Correlation between gestational age and level of epidermal growth factor binding in placental homogenates of male and female fetuses. Level of epidermal growth factor binding was determined from the ISIS-12 program after Scatchard transformation of binding data and was significantly correlated with fetal age in weeks of gestation when subjected to Kendall's rank correlation test.

cific epidermal growth factor binding after rapid debinding and rebinding was very similar in placental membranes from male and female fetuses (92% ± 17% and 90% ± 13%, respectively). Assuming any permanent denaturation of epidermal growth factor receptor during rapid debinding is identical between placental membranes of male or female fetuses, occupation of epidermal growth factor receptors by endogenous epidermal growth factor cannot explain the approximate twofold increases in epidermal growth factor binding observed in placental membranes of male fetuses compared with female fetuses.

Specific binding to preterm placentas. Titration of ^{125}I-labeled epidermal growth factor binding to homogenates of preterm placentas was very similar to that of homogenates obtained from term placentas. Nonspecific binding was linear in the range of the assay (mean $R^2 = 0.99$; $n = 11$) and represented approximately 60% of the total binding at high levels of ^{125}I-labeled epidermal growth factor. Specific binding plateaued in all 11 homogenates, and Scatchard transformations were curvilinear. Dissociation constants for low- and high-affinity sites were 560 ± 100 and 210 ± 100 pmol/L, respectively, and were in good agreement with half-saturation values obtained from titration curves. Dissociation constants were not significantly different ($p > 0.5$) between placentas of preterm male and female fetuses.

Kendall's rank order correlation test (Fig. 3) demonstrated a significant positive correlation of gestational age between 20 and 40 weeks with the level of receptors for epidermal growth factor in placentas from male fetuses ($n = 17$; $p < 0.002$; $T = 0.527$) as well as in placentas of female fetuses ($n = 18$; $p < 0.01$, $T = 0.404$).

Comment

Our results demonstrate that membranes from term placentas of normal male fetuses express significantly higher levels of epidermal growth factor binding than membranes from term placentas of normal female fetuses. This difference could not be explained by higher percent occupancy of epidermal growth factor receptors by endogenous epidermal growth factor in placental membranes from female placentas, since rapid debinding of epidermal growth factor followed by measurement of specific epidermal growth factor binding indicated very similar levels of recovery of epidermal growth factor binding compared to untreated membranes. It is interesting to note that Benveniste and Carson[12] also recently reported that epidermal growth factor binding was significantly higher in liver cell membranes prepared from male rats compared with female rats. Thus two tissues have shown sexual dimorphism of epidermal growth factor binding, that is, rat liver and human placenta. Presumably, the higher level of epidermal growth factor binding in the male placenta represents an important physiologic distinction in the magnitude or sensitivity of response to epidermal growth factor between placental cells of male and female fetuses.

We also observed a significant positive correlation between gestational age and epidermal growth factor binding during the second half of pregnancy (20 weeks

to term) for male and female placentas. Our results are in general agreement with those of Carson et al.[13] who reported that Scatchard analysis of levels of specific epidermal growth factor binding to placental membranes showed a significant positive correlation with advancing gestational age during the first and second trimesters (6 to 21 weeks) and at term. Lai and Guyda[2] also reported a significantly higher level of epidermal growth factor binding to cultures of placental cells established from a term placenta compared with that of placental cell cultures established from a placenta of a fetus of 15 weeks' gestation. Levels of epidermal growth factor binding were not correlated with sex of fetuses in either previous study.[2, 13]

The physiologic significance of the increase in level of epidermal growth factor binding during the first, second, and third trimesters of pregnancy suggested by our data and the data of Carson et al.[13] is not known. However, we have noted a peak of maternal urinary epidermal growth factor level around 20 weeks of gestation, followed by a rise near term (unpublished observation). Fluctuations in placental epidermal growth factor receptor level and maternal-fetal blood levels of epidermal growth factor would seem to suggest an important dynamic role of this factor and its receptor in pregnancy.

Regulation of epidermal growth factor receptor levels in the placenta is poorly understood. Placental epidermal growth factor receptors do undergo the expected acute down-regulation phenomenon observed with other cells when exposed to high levels of epidermal growth factor,[2] but further information regarding regulation of epidermal growth factor receptors in placenta cells is lacking. Regulation of epidermal growth factor receptor level has been studied more thoroughly in hepatocytes. Epidermal growth factor binding in membrane preparations from livers of hypothyroid rats was approximately 35% compared with that in euthyroid control animals, and treatment of hypothyroid animals with a single dose of triiodothyronine restored epidermal growth factor binding to near control levels.[14] Also, while epidermal growth factor exposure acutely induced down-regulation of epidermal growth factor receptors in nontransformed rat liver epithelial cells in vitro, epidermal growth factor also increased epidermal growth factor receptor messenger ribonucleic acid levels by threefold to fivefold, thus counterbalancing the acute down-regulation effect to some extent.[15]

Although the physiologic roles of epidermal growth factor and its receptor in the placenta are not clearly understood, the substantial levels of epidermal growth factor receptors in the placenta strongly imply their importance. The sexual dimorphism of epidermal growth factor receptor levels and the progressive increase in receptors during the second half of gestation reported here add additional complexities that need to be considered in developing models for their roles. It is interesting to speculate that epidermal growth factor and its receptors might be involved in one of the sexual dimorphic traits of newborn infants such as birth weight.[16] Additional data, however, are required to evaluate this possibility.

We thank Collin Osuamkpe for his help in the binding studies.

REFERENCES

1. Hock RA, Hollenberg MD. Characterization of the receptor for epidermal growth factor–urogastrone in human placenta membranes. J Biol Chem 1980;255:10731.
2. Lai WH, Guyda HJ. Characterization and regulation of epidermal growth factor receptors in human placental cell cultures. J Clin Endocrinol Metab 1984;58:344.
3. Benveniste R, Speeg Jr KV, Carpenter G, Cohen S, Linder J, Rabinowitz D. Epidermal growth factor stimulates secretion of human chorionic gonadotropin by cultured human choriocarcinoma cells. J Clin Endocrinol Metab 1978;46:169.
4. Huot RI, Foidart JM, Nardone RM, Stromberg K. Differential modulation of human chorionic gonadotropin secretion by epidermal growth factor in normal and malignant placental cultures. J Clin Endocrinol Metab 1981;53:1059.
5. Bahn RS, Speeg Jr KV, Ascoli M, Rabin D. Epidermal growth factor stimulates production of progesterone in cultured human choriocarcinoma cells. Endocrinology 1980;107;2121.
6. Rao CV, Carmen FR, Chegini N, Schultz GS. Binding sites for epidermal growth factor in human fetal membranes. J Clin Endocrinol Metab 1984;58:1034.
7. Lowry OH, Rosebrough NJ, Farr AL, Randal RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:263.
8. Savage CR, Cohen S. Epidermal growth factor and a new derivative. J Biol Chem 1972;247:7609.
9. Ramani N, Rao CV, Woost PG, Schultz GS. The presence of epidermal growth factor binding sites in the intracellular organelles of term human placenta. J Cell Sci 1986;84:19.
10. Thakur AK, Jaffe ML, Rodbard D. Graphical analysis of ligand binding systems: evaluation by Monte Carlo studies. Anal Biochem 1980;107:279.
11. Krupp MN, Connolly DT, Lane DM. Synthesis, turnover, and down-regulation of epidermal growth factor receptors in human A431 epidermoid carcinoma cells and skin fibroblasts. J Biol Chem 1982;257:11489.
12. Benveniste R, Carson SA. Binding characteristics of epidermal growth factor receptors in male and female rat liver cell membrane preparations. Mol Cell Endocrinol 1985;41:147.
13. Carson SA, Chase R, Ulep E, Scommegna A, Benveniste R. Ontogenesis and characteristics of epidermal growth factor receptors in human placenta. Am J Obstet Gynecol 1983;147:932.
14. Mukku R. Regulation of epidermal growth factor receptor levels by thyroid hormone. J Biol Chem 1984;259:6543.
15. Earp HS, Austin KS, Blaisdell J, et al. Epidermal growth factor stimulates EGF receptor synthesis. J Biol Chem 1986;261:4777.
16. Moltini RA, Stys SJ, Battaglia FC. Relationship of fetal and placental weight in human beings: fetal/placental weight ratios at various gestational ages and birth weight distributions. J Reprod Med 1978;21:327.

Immunohistochemical localization of epidermal growth factor receptor and myc oncogene product in human placenta: Implication for trophoblast proliferation and differentiation

Takeshi Maruo, M.D., and Matsuto Mochizuki, M.D.
Kobe, Japan

Cytologic localization of epidermal growth factor receptor and myc oncogene protein product in developing human placenta was analyzed by avidin/biotin immunoperoxidase techniques with a monoclonal antibody to epidermal growth factor receptor and an affinity purified polyclonal antibody to myc protein product. Epidermal growth factor receptor was found to be almost exclusively localized to syncytiotrophoblast, paralleling the immunohistochemical localization of human chorionic gonadotropin. Since epidermal growth factor has been shown to stimulate human chorionic gonadotropin and human placental lactogen production by cultured early placental tissue, the present finding that epidermal growth factor receptor was localized to mitotically inactive syncytiotrophoblast suggests a role for epidermal growth factor receptor in the induction of differentiated function of trophoblast rather than trophoblast proliferation. By contrast, myc protein product was found to be predominantly localized to cytotrophoblastic cells, paralleling the autoradiographic distribution of replicating cytotrophoblast identified by tritiated thymidine labeling of placental explant. A close similarity between the cytologic localization of myc protein product and tritiated thymidine labeling of placental explant suggests that myc protein expression is linked to trophoblast proliferation. Furthermore, immunohistochemical cellular levels of both epidermal growth factor receptor and myc protein product were most pronounced in early placenta and declined in term placenta. Thus myc protein product and epidermal growth factor receptor seem to play a crucial role in the induction of trophoblast proliferation and differentiation, respectively, during the development of human placenta. (AM J OBSTET GYNECOL 1987;156:721-7.)

Key words: Placental trophoblast, proliferation, differentiation, epidermal growth factor receptor, myc oncogene product

The trophoblast in early human placenta is composed of two distinct layers: an outer cell layer of multinuclear syncytiotrophoblast and inner cell layer of mononuclear cytotrophoblast. The cytotrophoblast displays highly proliferative and invasive properties very early in the first trimester. It is now evident that the syncytiotrophoblast is formed from the cytotrophoblast by a process of proliferation followed by cell fusion, and it displays little potential for proliferation.[1, 2] The syncytiotrophoblast is therefore regarded as mitotic end-stage cell derived from cytotrophoblast.

However, regulatory factors involved in processes of proliferation and differentiation of the placental trophoblast still remain to be elucidated. We have recently demonstrated that epidermal growth factor, a single chain polypeptide that is mitogenic for a variety of cultured cells, stimulates the production of both human chorionic gonadotropin and human placental lactogen by cultured early placental tissues,[2a] which suggests that epidermal growth factor may play a role in the induction of the differentiated function of trophoblast.

On the other hand, abundant evidence is now available that a number of the oncogenes of transforming viruses are related to normal growth factors and their receptors. Close similarity has been noted between platelet-derived growth factor and the translation product of v-sis oncogene[3] and between the cytoplasmic domain of epidermal growth factor receptor and the translation product of erb-B oncogene.[4] Of about 20 cellular oncogenes, myc oncogene is known to encode a protein localized to the nucleus of transformed cells. Several lines of recent evidence suggest a role for myc oncogene in cell proliferation.[5]

In the present study, attention was focused on the

From the Department of Obstetrics and Gynecology, Kobe University School of Medicine.
Supported by Grant in Aid for Scientific Research No. 60480368, 61570795 from the Japanese Ministry of Education, Science and Culture.
Received for publication July 11, 1986; revised September 3, 1986; accepted September 10, 1986.
Reprint requests: Takeshi Maruo, M.D., Department of Obstetrics and Gynecology, Kobe University School of Medicine, Kobe 650, Japan.

expression of epidermal growth factor receptor and myc oncogene product in normal human placenta; the cytologic localization of these two factors in developing human placenta observed by the immunohistochemical techniques with a monoclonal antibody to epidermal growth factor receptor and an affinity-purified polyclonal antibody to human myc product was discussed in relation to their possible physiologic roles in the development of human placenta.

Material and methods

Material. First-trimester placentas were obtained from patients undergoing elective abortions at 6 to 10 weeks of pregnancy. Midterm placentas were obtained at the time of elective abortion at 20 to 22 weeks of pregnancy, whereas term placentas were obtained after spontaneous term deliveries with no medical complications.

Immunohistochemical staining. Placental tissues obtained were fixed in 4% buffered neutral formalin, dehydrated and embedded in paraffin. Sections, 5 to 6 μm in thickness, were deparaffinized and followed by standard histologic techniques.

Immunohistochemical staining was performed by avidin/biotin immunoperoxidase techniques[6] with the use of a polyvalent immunoperoxidase kit (Omnitags, Lipshaw, Michigan), which can be used with any primary antibody produced in rabbit, sheep, mouse, or guinea pig. A mouse monoclonal antibody against mouse epidermal growth factor receptor (Oncor Inc., Gaithersburg, Maryland), an affinity-purified sheep polyclonal antibody against human myc translation product (Oncor Inc.), and an affinity purified rabbit polyclonal antibody against hCGβ-carboxyl terminal peptide (Takeda Chemical Industries, Osaka, Japan) were used, respectively, as the primary antibodies in this study.

Tissue sections were first bleached in 3% hydrogen peroxide to block endogenous peroxidase and covered with protein blocking agent to minimize nonspecific antibody binding. Sections were then incubated with the respective primary antibody for 60 minutes at room temperature. The anti–epidermal growth factor receptor antibody, anti-myc protein antibody, and anti–hCGβ-CTP antibody were diluted 1:50, 1:20, and 1:50 before use, respectively. The first incubation with primary antibody was followed by the second incubation with biotinylated polyvalent antibody and by the third incubation with avidin-horseradish peroxidase. Then, chromogenic reaction was developed by incubating with freshly prepared solution of tetrahydrochloride-diaminobenzidine and hydrogen peroxide. Sections were counterstained with Harris hematoxylin.

The following control procedures were undertaken to assure specificity of the immune reactions. Adjacent control sections were subjected to the same immunoperoxidase method with the exception that the primary antibodies to epidermal growth factor receptor, myc product, and hCGβ–carboxyl terminal peptide were respectively replaced by nonimmune murine IgG (Miles, Elkhart, Indiana), nonimmune sheep IgG (Miles) and nonimmune rabbit IgG (Miles). Additional control was prepared by the omission of the biotinylated polyvalent antibody. In the above-mentioned controls, no positive staining was observed.

Autoradiography of placental explants exposed to tritiated thymidine. Fresh sterile placental tissues were rinsed with ice-cold Hanks balanced salt solution, and placental villous tissues dissected free of chorionic membranes were cut into approximately 1 mm^3 explants.

The placental explants were placed on filter papers within a plastic plate to which McCoy's 5a medium (Grand Island Biological Co., Grand Island, New York) containing 1 μCi/ml of tritiated thymidine (New England Nuclear, Boston, Massachusetts) was added. The explants were incubated at 37° C for 2 hours in an atmosphere of 95% air and 5% carbon dioxide. After the pulse incubation, the placental explants were subjected to autoradiography by a modified method of Kopriwa and Leblond.[7] Briefly stated, paraffin-embedded sections, 5 μm in thickness, were deparaffinized, hydrated, and dipped in undiluted Kodak liquid emulsion type NTB-2. After partial drying they were exposed at 4° C for 2 weeks in sealed Bakelite boxes containing Drierite and then developed in Kodak D-19 developer. The developed sections were stained with Harris hematoxylin.

Results

Cytologic localization of epidermal growth factor receptor. Fig. 1, *A*, shows the immunohistochemical staining for epidermal growth factor receptor in very early first-trimester placenta obtained at 6 weeks of pregnancy. Prominent staining with anti–epidermal growth factor receptor antibody was almost exclusively localized to the syncytiotrophoblasts. The immunostaining for epidermal growth factor receptor of the syncytiotrophoblasts was apparent not only on the cellular surface but also on the cytoplasma. Neither cytotrophoblastic cells nor interstitial cells of placental villi showed any appreciable staining for epidermal growth factor receptor.

The intensity of the immunostaining for epidermal growth factor receptor of placental cells varied over the course of pregnancy. The syncytiotrophoblasts of early first-trimester placenta demonstrated the most pronounced staining for epidermal growth factor receptor

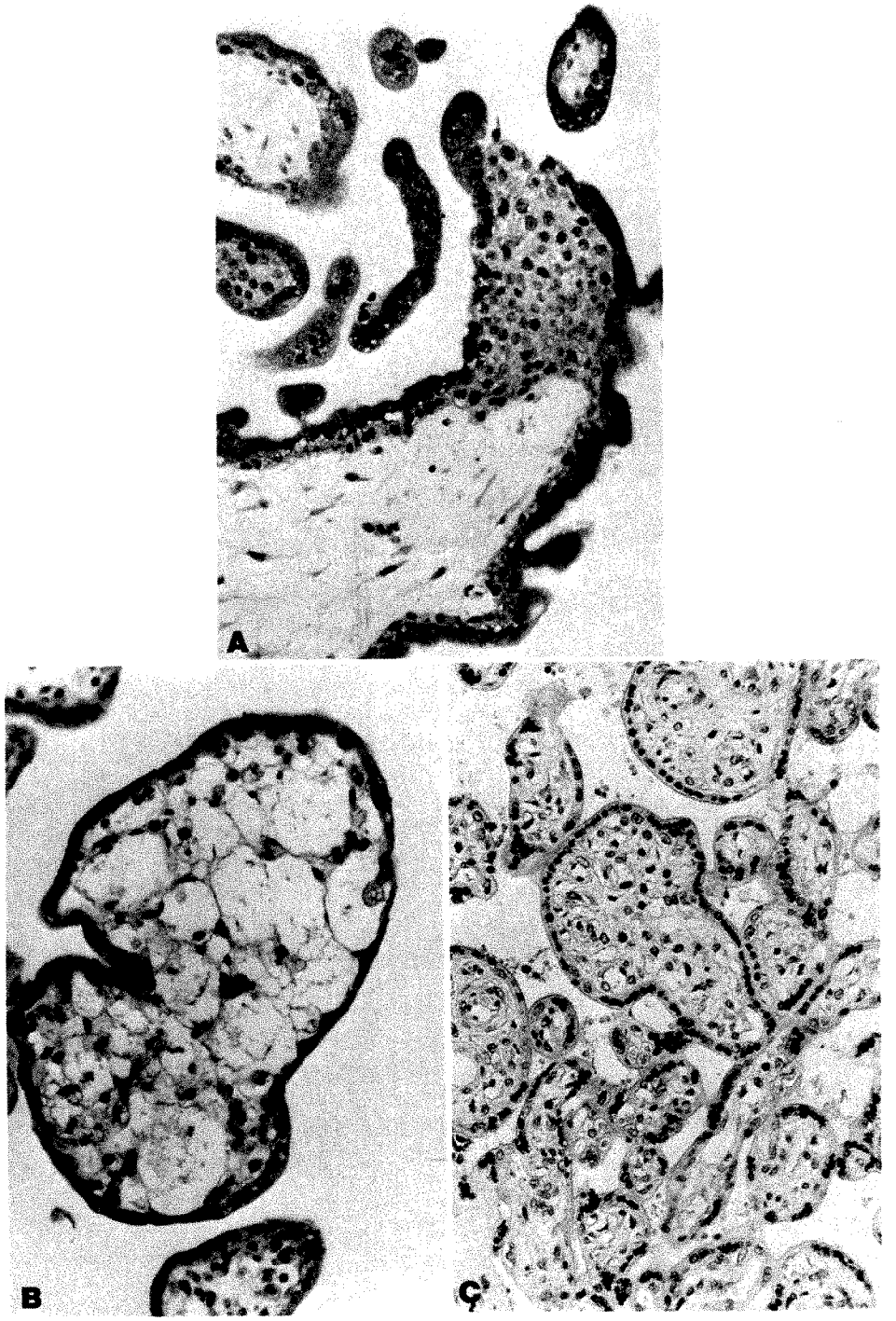


Fig. 1. Immunohistochemical localization of epidermal growth factor receptor in placental tissue sections over the course of pregnancy. Formalin-fixed paraffin-embedded sections of a 6-week placenta *(A)*, 22-week placenta *(B)*, and 40-week placenta *(C)* were stained by the avidin/biotin immunoperoxidase method with a monoclonal antibody directed against epidermal growth factor receptor. Most pronounced staining for the receptor was found in the early placental syncytiotrophoblasts, while the cytotrophoblastic cells were negative for appreciable staining. The staining intensity for epidermal growth factor receptor declined with the advancing ages of pregnancy. The lighter staining of nuclei is the hematoxylin counter stain. (× 200.)



Fig. 2. Immunohistochemical localization of hCGβ–carboxyl terminal peptide in early first-trimester placenta. Formalin-fixed paraffin-embedded sections of a 9-week placenta were stained by the immunoperoxidase method with anti–hCGβ–carboxyl terminal peptide antibody which is absolutely specific for human chorionic gonadotropin. Syncytiotrophoblasts showed pronounced staining with anti–hCGβ–carboxyl terminal peptide antibody, whereas cytotrophoblasts were negative for the immunostaining for human chorionic gonadotropin. (×200.)

(Fig. 1, *A*). In the case of midterm placenta obtained at 22 weeks of pregnancy (Fig. 1, *B*), the staining intensity for epidermal growth factor receptor of the syncytiotrophoblast was less prominent compared to that of early placenta, and term placenta showed a faintly positive staining for epidermal growth factor receptor (Fig. 1, *C*).

The immunohistochemical distribution of epidermal growth factor receptor observed in early placenta was very similar to that of human chorionic gonadotropin. The immunostaining with anti–hCGβ–carboxyl terminal peptide antibody was exclusively localized to syncytiotrophoblasts of developing human placenta (Fig. 2).

Cytologic localization of myc protein product. Fig. 3, *A* shows the immunohistochemical staining for myc protein product in early first-trimester placenta obtained at 6 weeks of pregnancy. Prominent staining with anti–myc product antibody was observed in the nuclei of the cytotrophoblastic cells. Although scarce staining for myc product was also apparent in the nuclei of the interstitial cells, no appreciable staining for myc product was found in the nuclei of syncytiotrophoblasts.

The intensity of the immunostaining for myc product in placental cells varied over the course of pregnancy. The most pronounced staining for myc product was observed in the cytotrophoblasts of early placenta (Fig. 3, *A*). In case of midterm placenta obtained at 22 weeks of pregnancy (Fig. 3, *B*), the staining intensity for myc product in the placental cells was less compared to that of early placenta. Term placental cells displayed no appreciable staining for myc product (Fig. 3, *C*).

Tritiated thymidine uptake by placental explants. Fig. 4 shows the autoradiogram of early placental explant exposed to tritiated thymidine for 2 hours. There were abundunt uptakes of tritiated thymidine in the nuclei of the cytotrophoblastic cells as well as interstitial cells. Tritiated thymidine uptake by the syncytiotrophoblasts was not encountered.

Comment

Epidermal growth factor is a polypeptide originally isolated from mouse submaxillary gland and later from human urine.[8] Although mouse and human epidermal growth factors differ slightly with regard to amino acid composition, they both are known to compete for the same receptor and demonstrate similar biologic activities.[9] It is now evident that epidermal growth factor receptor, a 170,000 molecular weight glycoprotein, is present in a variety of cell types.[10] Because of the participation of epidermal growth factor receptor in growth control and because of the close relationship of epidermal growth factor receptor to the erb-B oncogene translation product, it seems likely that there is the link between oncogenic stimulation of a cell and its normal growth-regulatory mechanism.[5,11]

The data presented in this paper shows that immu-

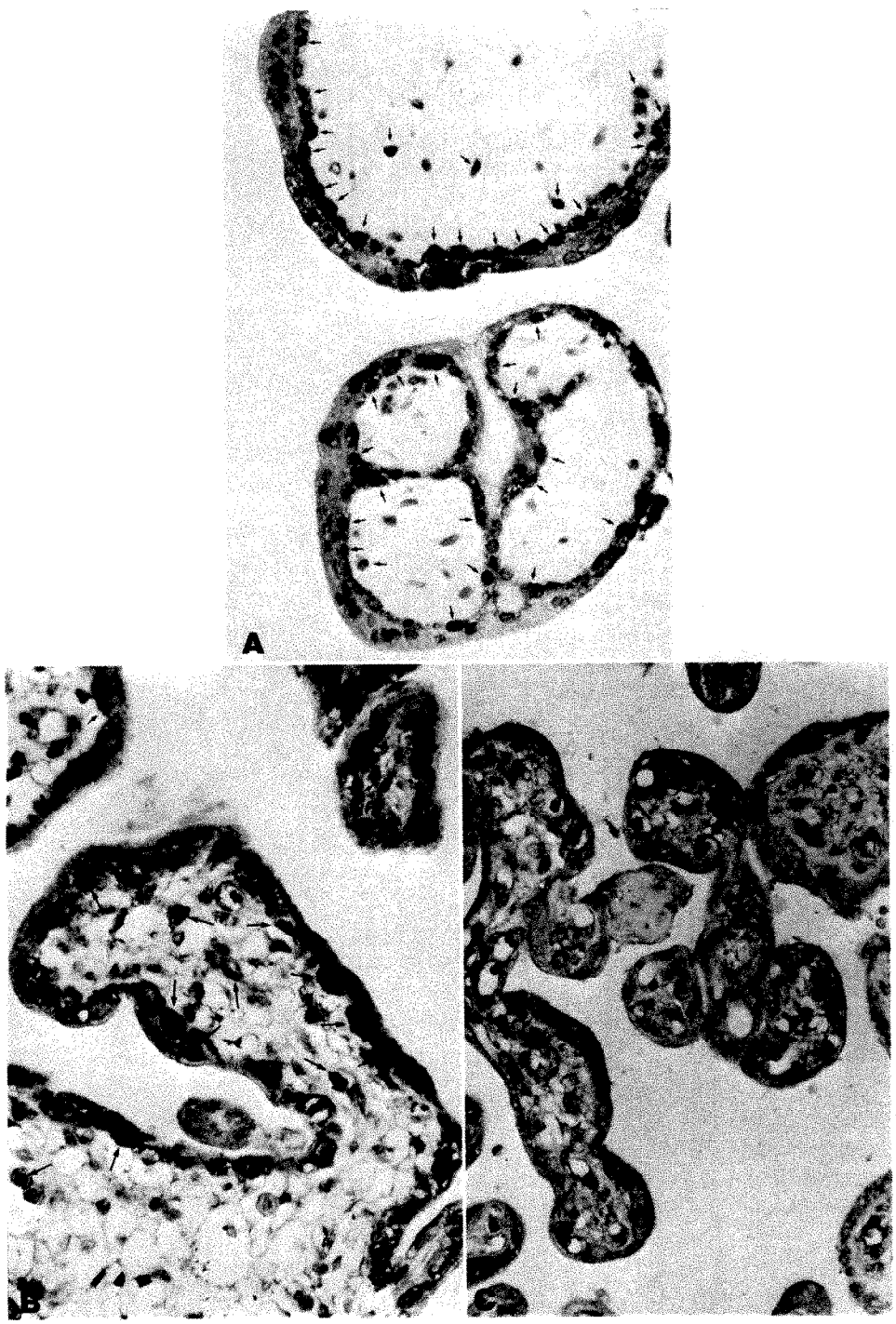


Fig. 3. Immunohistochemical localization of myc oncogene product in placental tissue sections over the course of pregnancy. Formalin-fixed paraffin-embedded sections of a 6-week placenta *(A)*, 22-week placenta *(B)*, and 40-week placenta *(C)* were stained by the avidin/biotin immunoperoxidase method with an affinity-purified antibody to myc protein product. Immunostaining for myc protein product was strongly positive in the nuclei of cytotrophoblasts and interstitial cells, whereas the nuclei of syncytiotrophoblasts were negative for the staining for myc protein product. The staining intensity markedly declined with the advancing ages of pregnancy. The lighter staining of nuclei is the hematoxylin counterstain. (×200.)

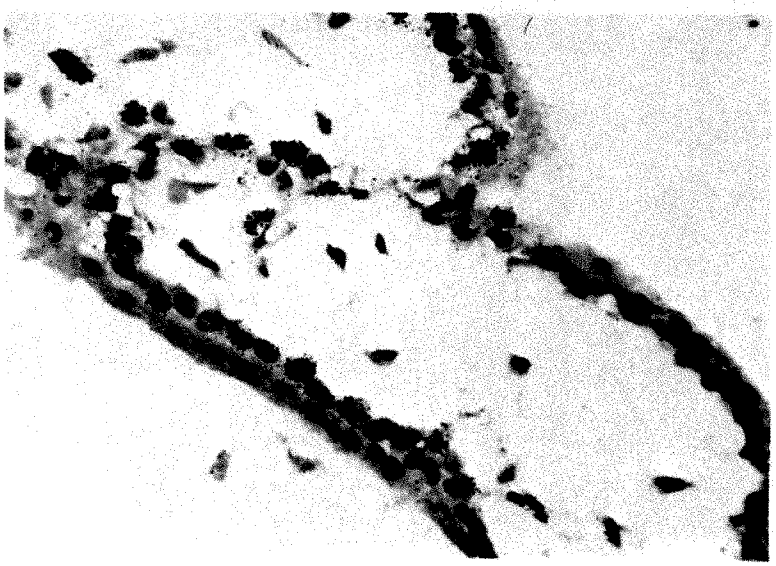


Fig. 4. Autoradiographic labeling of tritiated thymidine by early placental explants. Tissue explants from a 6-week placenta were exposed to tritiated thymidine (1 μCi/ml) for 2 hours. The formalin-fixed paraffin-embedded sections were subjected to autoradiography. Tritiated thymidine labeling was strongly positive in the nuclei of cytotrophoblasts, whereas there was no labeling of tritiated thymidine in syncytiotrophoblasts. (×400.)

nohistochemical cellular levels of epidermal growth factor receptor and myc oncogene product in human placenta vary considerably over the course of pregnancy and both the cellular levels are highest in early first-trimester placenta. We have also provided evidence that epidermal growth factor receptor in the placenta is almost exclusively localized to syncytiotrophoblast and is negative in cytotrophoblast, whereas myc oncogene product is predominantly localized to cytotrophoblast and is negative in syncytiotrophoblast. The inverse relation of the expression of epidermal growth factor receptor and myc oncogene product in early placenta and a decline in the cellular expression levels of both epidermal growth factor receptor and myc oncogene product in midterm and term placenta are of great interest when considering their possible physiologic roles in the control of placental development.

The localization of epidermal growth factor receptor on the cellular surface and cytoplasma of the syncytiotrophoblast of human placenta paralleled the immunohistochemical distribution of human chorionic gonadotropin and humman placental lactogen. The present immunohistochemical finding is in agreement with the autoradiographic data reported with iodine 125–labeled epidermal growth factor by Chengi and Rao,[12] who demonstrated that syncytiotrophoblast cells contain the epidermal growth factor receptors and that these receptors are present not only in outer cell membrane but also in intracellular organelles of human placental syncytiotrophoblasts. The fact that the mitotically active cytotrophoblasts were negative for epidermal growth factor receptor expression suggests that epidermal growth factor and its receptor may not be involved in the control of multiplication of trophoblasts. Instead, epidermal growth factor and its receptor seem to play a role in the induction of differentiated function of trophoblast, since epidermal growth factor has been already found to stimulate the production and secretion of human chorionic gonadotropin, α-human chorionic gonadotropin, and human placental lactogen by cultured early placental tissues.[2a]

On the other hand, the immunohistochemical localization of myc oncogene product to the nuclei of cytotrophoblasts of early placenta paralleled the autoradiographic distribution of replicating cytotrophoblasts identified by tritiated thymidine labeling of early placental explants. This indicates that there is a close correlation between the expression of myc oncogene product and cytotrophoblastic proliferation. The present immunohistochemical finding is in good agreement with the data of Pfeifer-Ohlsson et al.,[13] who demonstrated prominent expression of myc oncogene in cytotrophoblasts of early human placenta by in situ hybridization to human myc probe. The nuclear site of myc oncogene product suggests a fundamental regulatory role for the myc oncogene in the cytotrophoblasts. Furthermore, the highly increased expression of the myc oncogene product in early placental cytotrophoblasts, which display very proliferative phenotype, also supports a possibility that the protein product encoded by myc oncogene may play a crucial role in the trophoblast proliferation.

In this context, remarkable changes in the morphologic structure of developing human placenta are of interest. Although the trophoblast in early placenta is composed of two distinct layers, syncytiotrophoblast and cytotrophoblast, there is a steady reduction in the number of cytotrophoblastic cells as the placenta develops.[14] The cytotrophoblastic cells become much less prominent during the second trimester and in some villi appear to be absent during the third trimester. These morphologic changes of developing human placenta seem to be influenced by the expression pattern of myc oncogene.

Recently Goustin et al.[15] have reported that cultured cytotrophoblasts responded to exogenous platelet-derived growth factor by activation of the myc oncogene expression, suggesting that platelet-derived growth factor is also involved in the stimulation of cytotrophoblast proliferation. At the present time, however, it is not clear yet whether the induction of myc oncogene expression is a sufficient event for trophoblast proliferation. Nevertheless, on the basis of the results reported here it is apparent that myc oncogene product and epidermal growth factor receptor play a physiologic role independently in the induction of proliferation and differentiation of human placenta.

REFERENCES

1. Gerbie AB, Hathaway HH, Brewer JI. Autoradiographic analysis of normal trophoblastic proliferation. AM J OBSTET GYNECOL 1968;100:640.
2. Kliman HJ, Nestler JE, Sermasi E, Sanger JM, Straus JF. Purification, characterization, and in vitro differentiation of cytotrophoblasts from human term placentae. Endocrinology 1986;118:1567.
2a. Maruo T, Matsuo H, Hayashi M, Mochizuki M. Induction of differentiated trophoblast function by epidermal growth factor. J Clin Endocrinol Metab 1987 (in press).
3. Johnsson A, Heldin CH, Wasteson A, et al. The c-sis gene encodes a precursor of the B chain of platelet-derived growth factor. EMBO J 1984;3:921.
4. Downward J, Yarden Y, Mayes, E, et al. Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequence. Nature 1984;307:521.
5. Land H, Parada LF, Weinberg RA. Cellular oncogenes and multistep carcinogenesis. Science 1983;222:771.
6. Giorno R. A comparison of two immunoperoxidase staining methods based on the avidin/biotin interaction. Diag Immunol 1984;2:161.
7. Kopriwa BM, Leblond CP. Improvements in the coating technique of radioautography. J Histochem Cytochem 1962;10:269.
8. Cohen S. Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the new-born animal. J Biol Chem 1962;237:1555.
9. Carpenter G, Cohen S. Biological and molecular studies of the mitogenic effects of human epidermal growth factor. In: Papaconstantinou J, Rutter WJ, eds. Molecular control of proliferation and differentiation. New York: Academic Press, 1978:292.
10. Adamson EP, Rees AR. Epidermal growth factor receptors. Mol Cell Biochem 1981;34:129.
11. Xu YH, Richert N, Ito S, Merlino T, Pastan I. Characterization of epidermal growth factor receptor gene expression in malignant and normal human cell lines. Proc Natl Acad Sci USA 1984;81:7308.
12. Chengi N, Rao ChV. Epidermal growth factor binding to human amnion, chorion, decidua, and placenta from mid and term pregnancy: quantitative light microscopic autoradiographic studies. J Clin Endocrinol Metab 1985; 61:529.
13. Pfeifer-Ohlsson S, Goustin AS, Rydnert J, et al. Spatial and temporal pattern of cellular myc oncogene expression in developing human placenta: implications for embryonic cell proliferation. Cell 1984;38:585.
14. Hamilton WJ, Hamilton DV. Development of the human placenta. In: Philipp EE, Barnes J, Newton M., eds. Scientific foundations of obstetrics and gynecology, London: Heinemann, 1977:292.
15. Goustin AS, Betsholtz C, Pfeifer-Ohlsson S, et al. Coexpression of the sis and myc protooncogenes in developing human placenta suggests autocrine control of trophoblast growth. Cell 1985;41:301.

Circannual rhythms in steroid receptor concentration in gynecologic and breast cancers

Joseph T. Chambers, Ph.D., M.D., Roberta Lawrence, M.P.H., Neil MacLusky, Ph.D., Arnold Eisenfield, M.D., and Peter E. Schwartz, M.D.

New Haven, Connecticut

Both estrogen and progestin receptor concentrations vary in a predictive temporal pattern for breast cancer; in epithelial ovarian cancer a temporal rhythmic pattern is suggested for estrogen receptor concentration only. No pattern for either receptor is seen for endometrial cancer. (AM J OBSTET GYNECOL 1987;156:728-9.)

Key words: Circannual rhythms, steroid receptor concentration, breast cancer, ovarian cancer, endometrial cancer

The influence of time and season on biologic parameters is well established; however, the possible impact on oncology has only recently been noted and explored. The concentration of estrogen receptors in breast cancer has been shown to have a temporal predictive value along a 1-year scale and decreased toxicities have been established with various diurnal schedulings of chemotherapeutic drugs.[1] We investigated in a population of patients with histologically confirmed primary breast cancer, epithelial ovarian cancer, and endometrial cancer the concentration of estrogen and progestin receptor values in the tumors as a function of month of the year.

Material and methods

Estrogen and progestin receptor concentrations were determined by binding techniques with the use of radioactive steroid ligands as described elsewhere.[2] Receptor concentrations were determined in 859 tumor samples obtained from postmenopausal women with primary breast cancer, in 105 tumor samples from postmenopausal women with epithelial ovarian cancer, and in 159 tumor samples from postmenopausal women with endometrial cancer. All samples were obtained between October 1976 and September 1985. Statistical analysis was done with the use of a *t* test with Bonferroni correction for multiple comparisons and an analysis of variance to test the fit of the cosine curve to the data.

From the Yale University School of Medicine.

Received for publication August 6, 1986; accepted September 10, 1986.

Reprint requests: Joseph T. Chambers, Ph.D., M.D., Yale University School of Medicine, 333 Cedar St., P. O. Box 3333, New Haven, CT 06510.

Results

We confirmed a previous report of a circannual rhythm in breast cancer estrogen receptor concentrations. The mean femtomoles of cytosol estrogen and progestin receptor per milligram of cytosol protein (±SEM) are plotted in Fig. 1 for the breast cancer samples.

The mean values for the period October through March were significantly higher than the mean values for the period April through September ($p = 0.012$) with a peak in December. The same trend was evident although slightly less significant when the zero values were eliminated, suggesting that the group of patients who had zero estrogen receptor values was not different from those who had positive receptor values. An analysis of variance showed that the amplitude of the fitted cosine curve was significantly higher in the winter months than in the summer months with a variation of 46%. Progestin receptor concentrations in the breast tumors showed temporal variations of a predicted pattern where the cosine fit was again significant ($p = 0.008$) (Fig. 1).

Estrogen receptor concentrations in epithelial ovarian cancer showed a seasonal variation with a peak in February through May and a trough in June through January; however, this was not statistically significant. The actual peak was bimodal in February and May (Fig. 1). There were only two ovarian cancer patients with zero estrogen receptor values so no separate analysis was executed on this set of data. There was no significant seasonal curve fit for the progestin receptor concentrations in the epithelial ovarian cancer samples.

Analysis showed that no significant cosine curve could be fitted to the estrogen or progestin receptor concentrations along a time series for the endometrial cancer data.

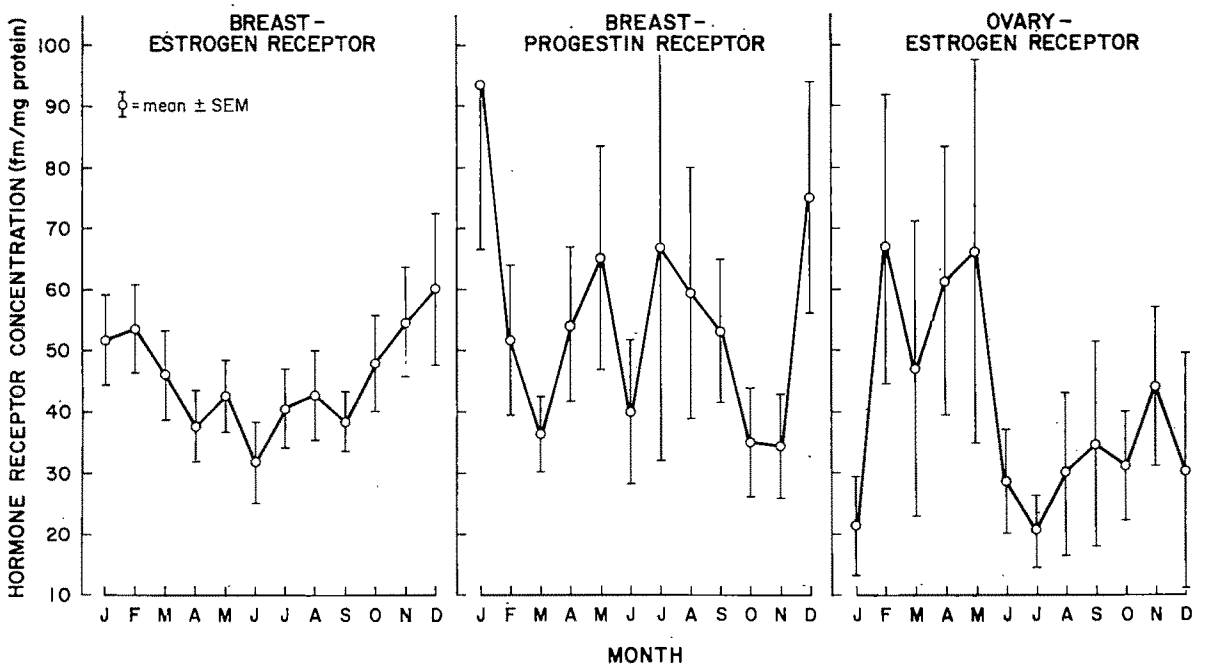


Fig. 1. Steroid receptor concentrations in breast and ovarian cancers as a function of the month.

A test for linear trend was included in all models that resulted in a significant negative trend during the period of years for all cancer types ($p = 0.001$ in breast cancer, $p = 0.0035$ in ovarian cancer, and $p = 0.0147$ in endometrial cancer). The reason for this overall negative trend remains uncertain. Perhaps there were population differences in the women over the years. A more likely possibility, however, is that the effect may be due to improvements in the receptor assay designed to minimize the contribution from low-affinity binding components. These modifications, which have been detailed previously,[2] may have given rise to the observed negative trend as a result of a slight overestimation of receptor concentrations in the earlier assays (1976 to 1979). Nevertheless, the predictive patterns noted above remain valid, since the same circannual trends are apparent irrespective of the years in which the data were obtained.

In conclusion, our observations confirm a temporal predictive pattern of estrogen receptor concentrations in breast cancer with a trough in April and a peak in December and also show a similar pattern for progestin receptor concentrations. However, in epithelial ovarian cancer, we observed a different seasonal pattern, the peak value for the estrogen receptor values being in the early part of the year compared with the trough in the midyear, but this was not statistically significant. The influence of the environment from a temporal standpoint may need to be further explored in determining the results from current treatment protocols in oncology.

REFERENCES

1. Hrushesky WJM. The clinical application of chronobiology to oncology. Am J Anat 1983;168:519-43.
2. Schwartz PE, Livolsi VA, Hildreth N, Macluskey NJ, Naftolin F, Eisenfeld AJ. Estrogen receptors in ovarian epithelial carcinoma. Obstet Gynecol 1982;59:229-38.

An improved human tumor stem cell assay in ovarian cancer

Peter G. Rose, M.D., Antoinette Koolemans-Beynen, M.S., John G. Boutselis, M.D., John P. Minton, M.D., Ph.D., and George E. Milo, Ph.D.
Columbus, Ohio

To evaluate the effect of improved growth rates of ovarian cancers in the human tumor stem cell assay and its value in predicting clinical chemotherapy response, we studied 59 assays in 54 patients. A total of 81.6% of solid specimens and 85.7% of ascites specimens were successfully cultured and yielded an overall growth rate of 82.9%. Simultaneous primary and metastatic cultures were concordant for chemosensitivity in 80% (n = 16). The patients were evaluated for previous chemotherapy, residual volume of tumor, histologic type, and grade, and these were not statistically different between clinical responders and nonresponders. In vivo–in vitro correlations were made in 27 patients and yielded a predictive response of 13% and predictive resistance of 86% at 70% colony inhibition and 31% and 71% at 50% colony inhibition. Improved growth rates therefore did not result in better predictive correlations. The reported experience in ovarian cancer is summarized and the current status of the human tumor stem cell assay is reviewed. (Am J Obstet Gynecol 1987;156:730-4.)

Key words: Stem cell assay, chemotherapy, ovarian cancer

The development of effective chemotherapy remains the most significant advance in the treatment of ovarian cancer in the last three decades. However, until recently, the selection of chemotherapeutic agents has been based on clinical experience. The development of the in vitro soft agar assay system in 1955[1] and its clinical application to human tumors in 1972[2] have suggested new avenues for the selection of a chemotherapeutic agent for patients on an individual basis determined by in vitro sensitivity. In addition to its ability to indicate specific tumor sensitivity, the assay could potentially save valuable time that would be lost on ineffective treatment. It also opens the possibility of in vitro testing of new chemotherapeutic drugs on human tumor cells before clinical application.

Numerous clinical reports suggest an in vitro–in vivo sensitivity correlation of 40% to 88% and an in vitro–in vivo resistance correlation of 90% to 100%.[3-6] In most of these studies, success rates in establishing growth in soft agar ranges from 25% to 50%.[7] The Ohio State University Clonogenic Assay Laboratory, established in 1981, has performed more than 900 human tumor stem cell assays with a variety of tumors. The modified procedure has resulted in an improved success rate of more than 80%. The purpose of this study was to investigate correlation of clinical and assay data in the presence of these improved culture conditions. The data presented in this paper include the results of 59 successful assays on ovarian tumor specimens, representing 54 patients. A retrospective in vivo–in vitro correlation is reported.

From the Departments of Obstetrics and Gynecology, Surgery, and Physiological Chemistry and the Comprehensive Cancer Center of The Ohio State University.
Received for publication June 5, 1986; revised September 22, 1986; accepted October 20, 1986.
Reprint requests: Peter G. Rose, M.D., 517 Means Hall, 1654 Upham Dr., Columbus, OH 43210.

Material and methods

Single cell preparation. Ovarian carcinoma specimens were obtained from patients from the following Columbus and area hospitals: The Ohio State University Hospitals (n = 42), Riverside Methodist Hospital (n = 4), Grant Hospital (n = 4), Mansfield General Hospital (n = 3), and Mt. Carmel West Hospital (n = 1) and assayed according to a modified procedure of the human tumor stem cell assay described by Hamburger and Salmon.[2] Briefly, the specimens were collected in medium under sterile conditions, transported to the laboratory, and processed within 24 hours. The tissue was minced and washed two times in minimum essential medium (K. C. Biological Inc., Lenexa, Kansas) containing 5% fetal bovine serum (Hyclone, Sterile Systems, Logan, Utah) and gentamicin (50 μg/ml). The tissue fragments were transferred into a Cellector sieve (Bellco, Vineland, New Jersey) equipped with a triple screen (120 mesh) and further minced with scalpels. Optimal viability was ensured by repeated rinsing with minimum essential medium. The resultant single cell suspension was centrifuged at 750 × g and then resuspended in minimum essential medium and a cell count taken. Cell density was adjusted to 1.5×10^6 cells per milliliter.

Ascites specimens were collected in sterile containers without heparin and the cells were pelleted by centrifugation at 750 × g. The cell pellet was resuspended in minimum essential medium and transferred to a Cellector sieve and processed as above. If a specimen did not yield a sufficient number of cells to complete an

Table I. Results of in vitro chemosensitivity tests

Drug	n	>50% inhibition		>70% inhibition	
		n	%	n	%
Adriamycin	73	10	14	4	5
Cisplatin	71	12	17	7	10
Methotrexate	72	14	19	4	6
Vinblastine	69	11	16	5	7
5-Fluorouracil	47	4	9	1	2
Dihydroxyanthracenedione	70	14	20	3	4
VP-16	32	3	9	2	6
Melphalan	16	3	19	0	0
Hexamethylmelamine	13	0	0	0	0
Mitomycin C	13	0	0	0	0
Aclacinomycin	24	5	21	2	8

assay, intermediate short-term monolayer cultures were set up in an effort to increase cell count.

Drug incubation. The following chemotherapeutic agents were prepared to achieve final concentrations equivalent to peak plasma concentrations as calculated by Alberts and Chen[8] against ovarian tumors: (1) Adriamycin, 0.04 μg/ml; (2) cisplatin, 0.2 μg/ml; (3) 5-fluorouracil, 6.0 μg/ml; (4) methotrexate, 100.0 μg/ml; (5) vinblastine, 6.0 μg/ml; (6) VP-16, 3.0 μg/ml; (7) aclacinomycin, 0.1 μg/ml; (8) dihydroxyanthracenedione, 0.1 μg/ml; (9) melphalan, 1.0 μg/ml; (10) hexamethylmelamine, 1.0 μg/ml; (11) mitomycin C, 1.0 μg/ml.

One milliliter of cell suspension and 0.35 ml of minimum essential medium were added to each test tube containing 0.15 ml of the appropriate 10× drug solution. The cell-drug mixture was gently stirred in a vortex mixer and then incubated for 1 hour at 37° C and 4% carbon dioxide. Two tubes containing 0.15 ml of phosphate-buffered saline solution were included for controls.

Media preparation and cell plating. Difco Bacto-Agar (Difco, Detroit, Michigan) was washed four times in demineralized water and brought into solution as previously reported.[9] Feeder layer and plating media were prepared according to the protocol of the Southwest Oncology Group, except that the ratio used was 2:1.[10]

At the conclusion of the 1-hour drug exposure period, the cells were washed by adding 5 ml of minimum essential medium to each drug incubation tube and the cells were removed by centrifugation. The supernatants were aspirated and replaced with 0.3% agar–plating medium mixture. The cells were dispersed by trituration and layered on top of each 0.5% agar feeder layer in 60 mm plastic Petri dishes as previously described.[9] Each experiment was performed in triplicate. Cultures were examined on day 0, and all clumps were identified for subtraction from the final colony count. In our laboratory, cell clumps were rare and occurred at a frequency of <1 per 50,000 cells plated. After 12 to 20 days of incubation at 37° C and 4% carbon dioxide in a humidified atmosphere, the formed colonies were counted with an inverted phase-contrast microscope. Percent inhibition of tumor colony formation was calculated with the following formula:

$$\text{Percent inhibition} = 1 - \frac{\text{number of colonies exposed}}{\text{number of colonies in control}} \times 100\%$$

A successful assay would contain at least 25 colonies in the control plates, each measuring ≥60 μm in diameter, or containing no less than 30 cells.[10]

Clinical evaluation and in vitro–in vivo correlation. Pathology slides of all patients were reevaluated and tumors were graded according to the classification of the International Federation of Gynecology and Obstetrics. The clinical records were reviewed and patients were classified as complete responders, partial responders, or nonresponders on the basis of objective criteria for tumor response defined by the World Health Organization. Patients were excluded if they failed to receive at least two courses of a chemotherapeutic agent for which they had been tested. Thus clinical and in vitro data for 27 patients could be correlated. Percent inhibition of tumor colony formation in vitro was analyzed at both >50% and at >70% inhibition levels as reported elsewhere (see Table III). Statistical analyses were completed with a χ^2 test.

Results

Eighty-four ovarian specimens were submitted from November 1981 through September 1985. Nine specimens had inadequate cell counts for soft agar chemosensitivity testing, but one was salvaged by intermediate culturing as described. The 76 specimens tested consisted of 60 solid tumors and 16 ascites specimens. Two solid tumors (3.3%) and two ascites specimens (12.5%) could not be evaluated because of bacterial contamination. Subsequent to tissue processing and plating, fungal contamination occurred in <4% of the total 2161 Petri plates. Clonogenic assays were successful in 81.6% of the solid specimens and 85.7% of

Table II. In vitro–in vivo correlation in patients with Stage III or IV ovarian cancer

	Previous therapy	*Residual tumor (cm)*	*Histologic type*	*Grade*	*Inhibition (≥50%/>70%)*
Complete clinical response					
M. G.	–	<2	Epithelial	3	–/–
G. E.	–	<2	Epithelial	3	–/–
Partial clinical response					
S. M.*	–	<2	Epithelial	3	–/+
R. F.*	–	<2	Epithelial	3	–/–
J. G.*	–	>2	Epithelial	3	–/–
B. T.*	–	<2	Epithelial	2	+/+
M. H.	–	<2	Epithelial	3	–/–
M. H.*	–	<2	Epithelial	3	+/–
H. B.*	+	<2	Epithelial	2	+/–
J. E.	+	<2	Epithelial	2	–/–
M. D.*	–	>2	Epithelial	2	–/–
K. P.*	–	<2	Germ cell		–/–
J. C.*	–	<2	Epithelial	2	–/–
No response					
M. P.*	–	<2	Epithelial	3	+/–
J. E.*	+	<2	Epithelial	3	–/–
H. C.	+	<2	Epithelial	2	–/–
M. D.*	–	<2	Epithelial	3	–/–
C. S.*	+	<2	Epithelial	3	–/+
T. R.*	–	>2	Epithelial	3	–/–
L. T.*	–	<2	Epithelial	3	–/–
F. M.*	+	<2	Epithelial	2	–/–
R. S.*	+	<2	Epithelial	2	–/–
D. K.*	–	<2	Epithelial	2	–/–
S. M.*	–	<2	Epithelial	2	–/+
D. E.*	–	>2	Epithelial	1	–/–
E. L.*	–	<2	Carcinosarcoma		–/–
M. W.*	–	>2	Epithelial	2	–/–

*Patients treated additionally with agents not assayed.

the ascites specimens tested, yielding an overall growth rate of 82.9%. Cloning efficiencies, the percentage of cells seeded that resulted in colony formation, ranged from 0.01% to 0.6% with a mean value of 0.15%.

The following chemotherapeutic agents were routinely tested: Adriamycin, cisplatin, methotrexate, vinblastine, 5-fluorouracil, and dihydroxyanthracenedione. Additional agents tested at the physician's request included VP-16, melphalan, hexamethylmelamine, mitomycin C, and aclacinomycin. Cyclophosphamide was not tested bcause it requires metabolic activation to be effective. The results are listed in Table I.

Fifty-nine successful assays were performed on specimens from 54 patients with primary ovarian cancer (50 epithelial, one granulosa, one dysgerminoma, and two carcinosarcoma).

Twenty-seven patients with Stage III or IV disease could be evaluated for response. Histologic type, grade, previous therapy status, clonogenic assay results, and clinical response are summarized in Table II. Evaluation correlating in vitro results with clinical outcome correctly predicted a positive response in 13% of the cases and a negative response in 86% at the 70% to 100% inhibition level. At the 50% to 100% inhibition level, these values were 31% and 71%. This distribution is not statistically significant.

The remaining 27 patients had to be excluded from the study. Seventeen of them received less than one course of a chemotherapeutic agent tested or were treated exclusively with agents not tested in the assay. Two patients who are currently receiving therapy are not evaluable. Also excluded were six patients who were clinically and radiologically without evidence of disease but who refused a second-look laparotomy. Two of these patients had recurrent disease 6 and 17 months after cessation of chemotherapy. Another patient with Stage IIA disease, treated with total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, and chemotherapy, could not be evaluated in the absence of measurable primary disease. Last, one patient was treated elsewhere for 19 months with clinical response but without objective evaluation until she died of disease.

Comment

Improved conditions in processing and culturing tumor tissue have resulted in successful growth rates for ovarian carcinomas of 82.7%. Significant improvements include standardized tissue collection procedures during operation by a tumor procurement service. Tissue dehydration is avoided by repeated rinsing of the tumor fragments with minimum essential me-

Table III. Ovarian cancer clonogenic assay in vitro–in vivo studies (literature summary)

	Positive in vitro and in vivo	*Positive in vitro, negative in vivo*	*Negative in vitro, positive in vivo*	*Negative in vitro and in vivo*	*Percent inhibition*
Alberts (Lancet 1980;2:340, N = 95)	13	8	1	73	50
Arbuck (Obstet Gynecol 1985;66:115, N = 28)	0	2	3	23	70
Moon (Cancer Chemother Pharmacol 1981;6:211, N = 90)	14	10	1	65	50
Alberts (Cancer Chemother Pharmacol 1981;6:279, N = 44)	8	3	0	33	50
Welander (Am J Obstet Gynecol 1983;147:188, N = 19)	9	1	0	9	50
Natale (Proc Am Assoc Cancer Res 1981;22:156, N = 28)	10	3	1	14	NR
Epstein (Cancer Chemother Pharmacol 1981;6:273, N = 12)	4	5	NR	NR	50
Wilson (Br J Cancer 1981;44:189, N = 15)	10	3	1	1	50
Current (N = 27)	4	3	9	11	50
Total assays (N = 355)	72	38	16	229	

Predictive of negative response = 93.4%. Predictive of positive response = 64.8%. NR: Not reported.

dium, and trauma is minimized by mincing the tissue with scalpels on a triple screen sieve. The low molecular weight polysaccharide toxicity of the agar[11] is reduced by repeated washing with demineralized water before autoclaving, and the modified ratio of two parts feeder layer to each one part plating layer favors growth. In spite of increased success rates in cloning the tumors, the assay's predictive power has not improved. A review of the literature presenting in vivo–in vitro correlation in ovarian cancer is summarized in Table III. Individual studies with success rates varying between 25% and 50%[7] show a high degree of correlation between negative human tumor stem cell assay results and clinical failure. Positive assay results, defined as either >50% inhibition or >70% inhibition, indicate predictability of clinical response in 60% of the cases. Cumulative results of 355 assays yield a predictive value of 93.4% for a negative human tumor stem cell assay and 64.8% for a positive assay (Table III).

Most correlation studies share common methodologic errors. Multiple assays performed on specimens from a single patient introduce serious sampling errors and compromise the statistical analysis of the data and the predictive value of the assay. Many patients have received agents not tested in the assay, notably cyclophosphamide (Cytoxan), which requires metabolic activation. In addition patients are often treated with multiple chemotherapeutic agents, and the significance of in vitro sensitivity to single agents, in contrast to multiple drugs, is not known. Alberts et al.[12] describes one of the few prospective studies reported in the literature to date. The in vitro directed therapy with one to three agents in which a 50% clinical response rate was achieved was significantly better ($p = 0.05$) than empiric therapy, although type of previous chemotherapy, volume of residual disease, and type of empiric therapy were not reported. Despite these limitations this study provides the greatest support for the human tumor stem cell assay. None of the authors has randomized previously untreated patients to chemotherapy directed by human tumor stem cell assay versus cisplatin and cyclophosphamide with or without Adriamycin with or without hexamethylmelamine.

Theoretical objections against the human tumor stem cell assay have recently been reviewed by Weisenthal and Lippman.[13] Principal arguments can be placed in four categories. First, cell cultures may represent only certain cell populations of the tumor. Resultant predictions of chemosensitivity may not take into account in vivo proliferation of cells that would not grow in the soft agar system. Arbuck et al.[14] described discrepancies between human tumor stem cell assay results of primary and metastatic specimens submitted simultaneously from the same patient. In our experience 16 paired observations (n = 6) indicate agreement in more than 80%. In one case the primary specimen did not grow, and only the metastatic tumor could be cloned successfully.

Second, in vitro drug concentrations and exposure times differ from in vivo drug protocols. Alberts et al.,[8] studying human subjects, established pharmacokinetic parameters for the most commonly used anticancer drugs and applied these findings to the clonogenic assay. In most studies drug exposure time in the assay is limited to 1 hour. Experiments with continuous exposure have been performed, but increases in colony formation inhibition were found to be minimal in most cases, possibly because of deactivation of the agents tested under culture conditions. In contrast to the human body, the assay is not a homeostatic system and drugs cannot be replenished nor can toxic waste products be eliminated.

Third, the percentage of tumor colony formation inhibition in vitro may not be relevant to clinical response. The effectiveness of a chemotherapeutic agent depends on the fraction of cell kill. From a mathematical viewpoint, effective agents must have a fractional cell kill of 99 to 99,999 (two to five log). The significance level for percent inhibition in the human tumor stem cell assay has been determined by in vitro–in vivo correlation,[8] but many factors affecting clinical response (previous chemotherapy, volume of residual disease, specific tumor histologic type and grade) must be controlled to make such a correlation valid. Tumor colony formation inhibition is not synonymous with cell killing, and cells may be arrested in a dormant yet viable state with potential for reactivation.

Finally, the assay ignores immunologic factors.

In this study clinical responders and nonresponders were not statistically different with respect to previous chemotherapy, treatment with drugs not tested in the assay, and volume of the residual tumor (see Table II). For disease evaluation all complete responders and 82% of the partial responders had a second-look laparotomy. Arbuck et al.[14] suggested that validation of the human tumor stem cell assay would require a prospective study of 500 patients to detect a 15% differential response rate. Most researchers agree that a single-agent human tumor stem cell assay study is desirable, but few clinicians are willing to risk negative results with single-agent therapy during an era in which combination therapy with drugs such as cisplatin, Cytoxan, Adriamycin, and hexamethylmelamine have become the standard[15, 16] with clinical response rates of 65% to 70%. The use of the human tumor stem cell assay seems more appropriate in patients who have failure of primary therapy with a regimen containing cisplatin, since second-line therapy in these patients is poor. Future prospective studies are indicated.

We would like to acknowledge the efforts of Dr. Kathryn Clausen for review of pathologic specimens. We would also like to acknowledge the following physicians for submitting specimens to our assay laboratory: Dr. J. Bell, Riverside Methodist Hospital; Dr. M. Cooperman, The Ohio State University Hospitals; Dr. D. Dewald, Mansfied General Hospital; Dr. G. Johnston, The Ohio State University Hospitals; Dr. L. Laufman, Mansfield General Hospital; Dr. T. Martin, The Ohio State University Hospitals; Dr. T. Nims, Grant Hospital; Dr. N. Teteris, The Ohio State University Hospitals; Dr. T. Williard, Mt. Carmel West Hospital, and Dr. F. Zuspan, The Ohio State University Hospitals.

REFERENCES

1. Puck TT, Marcus PI. A rapid method of viable cell titration and clone production with HeLa cells in tissue culture: use of X-radiated cells to supply conditioning factors. Proc Natl Acad Sci USA 1955;41:432-7.
2. Hamburger AW, Salmon SE. Primary bioassay of human tumor stem cells. Science 1977;197:461-3.
3. Von Hoff DD, Casper J, Bradley E, et al. Association between human tumor colony forming assay results and response of an individual patient's tumor to chemotherapy. Am J Med 1981;70:1027-32.
4. Alonso K. Human tumor stem cell assay: a prospective clinical trial. Cancer 1984;54:2475-9.
5. Fan D, Morgan LR, Schneider C, et al. Cooperative evaluation of human tumor chemosensitivity in the soft agar assay and its clinical correlation. Cancer Res Clin Oncol 1984;109:23-8.
6. Benard J, Riou G. Culture in vitro des cellules clonogenes de differentes tumeurs humaines: essais de chimiosensibilites. Bull Cancer (Paris) 1984;4:287-91.
7. Bradley EC, Issel BF, Hellman R. The human tumor colony forming chemosensitivity assay: a biological and clinical review. Invest New Drugs 1984;2:59-70.
8. Alberts DS, Chen HSG. Tabular summary of pharmacokinetic parameters relevant to in vitro drug assay cloning of human tumor stem cells. In: Salmon SE, ed. Cloning of human tumor stem cells. New York: Alan R Liss, 1980:351-9.
9. Schuller DE, Koolemans-Beynen AR, Libby DH, et al. The impact of nodal immunoreactivity in head and neck cancer. Laryngoscope 1985 (in press).
10. Soehnlen B, Young L, Liu R. Standard laboratory procedures for in vitro assay of human tumor stem cells. In: Salmon SE, ed. Cloning of human tumor stem cells. New York: Alan R Liss, 1930:331-8.
11. MacPherson IA. Soft agar techniques. In: Kruse PF, Patterson MK, eds. Tissue culture methods and applications. New York: Academic Press, 1973:276-80.
12. Alberts DS, Leigh S, Surwit EA, et al. Improved survival of patients with relapsing ovarian cancer treated on basis of drug selection following human tumor clonogenic assay. In: Salmon SE, Trent JM, eds. Human tumor cloning. Orlando: Grune & Stratton, 1984.
13. Weisenthal LM, Lippman ME. Clonogenic and non-clonogenic in vitro chemosensitivity assays. Cancer Treat Rep 1985;69:615-32.
14. Arbuck SG, Pavelic ZP, Piver MS, et al. Limitations of drug sensitivity testing in soft agar for clinical management of patients with ovarian carcinoma. Obstet Gynecol 1985; 66:115-9.
15. Ehrlich CE, Einhorn LH, Stehman FB, et al. Response, "second look" status and survival in stage III-IV epithelial ovarian cancer treated with cis-dichlorodiammine platinum II (cis-platinum), Adriamycin, and Cytoxan [Abstract]. Gynecol Oncol 1980;10:367-8.
16. Vogl SE, Berenzweig M, Kaplan BH, et al. The CHAD and HAD regimens in advanced ovarian cancer: combination chemotherapy including cyclophosphamide, hexamethylmelamine, Adriamycin and cis-dichlorodiammine-platinum (II). Cancer Treat Rep 1979;63:311-7.

Ontogeny of pituitary β-endorphin and related peptides in the human embryo and fetus

F. Facchinetti, M.D., A. R. Storchi, M.D., F. Petraglia, M.D., G. Garuti, M.D., and A. R. Genazzani, M.D.

Modena, Italy

In this study we evaluated the presence of proopiomelanocortin-related peptides (β-lipotropin, β-endorphin, and γ-endorphin) in five embryos (5 to 10 weeks of pregnancy) and 11 fetal pituitaries (15 to 25 weeks) by means of high-performance liquid chromatography coupled with specific radioimmunoassays. Tissues were collected at laparotomy for ectopic pregnancy (five embryos) or after spontaneous (seven) or prostaglandin-induced (four) abortion. β-Endorphin and β-lipotropin were present starting at the seventh week of pregnancy while γ-endorphin appeared only in the second trimester. During embryonic life opioid activity was limited to the cephalic portion. The three peptides, but little, if any, acetylated 1-31 β-endorphin, were recognized in the fetal pituitary throughout the second trimester, at which time β-lipotropin and β-endorphin showed constant values in spite of increasing γ-endorphin concentrations. β-Lipotropin was the predominant peptide in both embryonic and fetal life. In conclusion, the three peptides related to proopiomelanocortin were expressed from the precursor at different times throughout development. By the beginning of the second trimester the pituitary processing of proopiomelanocortin is similar to that of adult life and the functional activity of the anterior lobe seems to prevail over that of the "fetus-related" neurointermediate lobe around the twenty-fifth week of pregnancy. (Am J Obstet Gynecol 1987;156:735-9.)

Key words: Embryo, anterior pituitary, neurointermediate lobe, β-endorphin, β-lipotropin, γ-endorphin

The activity of the proopiomelanocortin-related opioid system undergoes important changes during pregnancy as suggested by the increased adrenocorticotropic hormone and β-endorphin levels in the maternal circulation[1] and by the changing number and affinity of naloxone binding sites in uterine rat membranes.[2] In addition to the maternal pituitary and placenta, whose opioid secretory ability has been demonstrated,[3] the fetal pituitary is believed to contribute to the increase in opioid peptide levels.

Immunohistochemical data indicate the presence of adrenocorticotropic activity from the seventh week of pregnancy[4] while no β-lipotropin, another proopiomelanocortin-related peptide, was found. On the other hand, Begeot and Dubois[5] recently reported the coexistence of several proopiomelanocortin-related peptides in fetal pituitary corticotropes.

Biochemical studies of opioid ontogeny in human material are scanty and contradictory. Some authors[6] reported a large amount of β-endorphin in the fetal pituitary, and others[7] described the presence of β-lipotropin but were unable to demonstrate authentic β-endorphin in the 11 to 19 weeks' gestational age range. Moreover, with the use of sophisticated techniques it has been ascertained that the fetal pituitary does not contain true α-melanocyte–stimulating hormone, as reported several years ago by Silman et al.,[8] but its desacetylated form, adrenocorticotropic hormone 1-13 amide.[9] More recently, the presence of α-endorphin and γ-endorphin, two N-terminal derivatives of β-endorphin (1-16 and 1-17, respectively), has been verified in the human pituitary gland.[10]

In this study therefore we evaluated β-lipotropin, β-endorphin, and γ-endorphin in embryos and fetal pituitaries up to the beginning of the third trimester by means of high-performance liquid chromatography coupled with specific radioimmunoassays.

Material and methods

Tissue collection. Five embryos at 5 to 10 weeks of pregnancy were obtained at operation in ectopic pregnancies. The smallest embryo had a rostral-caudal length of 10 mm, ascribed to a gestational age of 4.7 weeks. In two specimens, the caudal portion was divided from the rostral one and processed separately.

Eleven whole pituitaries were collected from fetuses between 15 and 25 weeks of pregnancy. Seven of the 11 fetuses were spontaneously aborted, and four were delivered after a prostaglandin induction of labor performed because of rubella infection. Every tissue was

From the Department of Obstetrics and Gynecology, University of Modena.

This work has been partly supported by CNR P.F. "Medicina Preventiva e Riabilitativa" SP7 and SP8.

Received for publication June 12, 1986; revised July 18, 1986; accepted September 2, 1986.

Reprint requests: Dr. F. Facchinetti, Clinica Ostetrica e Ginecologica, Via del Pozzo 71, 41100 Modena, Italy.

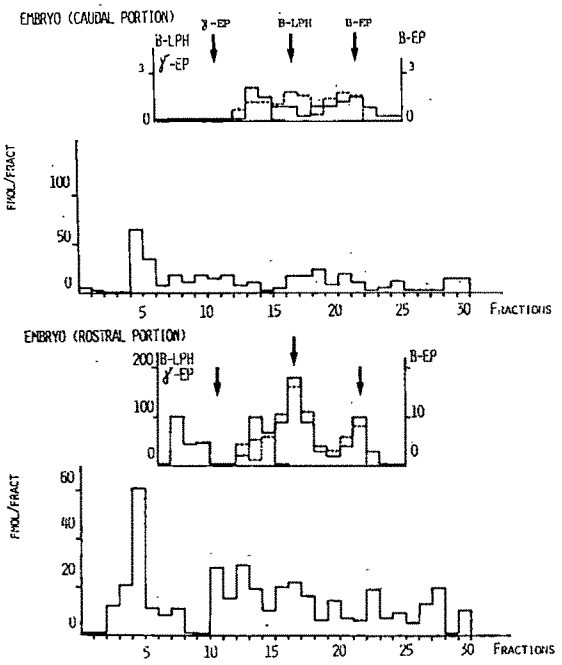


Fig. 1. Chromatogram of β-endorphin–like immunoreactivity and specific γ-endorphin *(γ-EP)*, β-lipotropin *(B-LPH)*, and β-endorphin *(B-EP)* immunoreactivity in caudal and rostral portions of a 7-week-old embryo. *Arrows* indicate elution of respective standard peptide. In the *small panel, solid line* on the left refers to *γ-EP* immunoreactivity; those on the right, to *B-EP* immunoreactivity; and *dotted line* refers to *B-LPH* immunoreactivity.

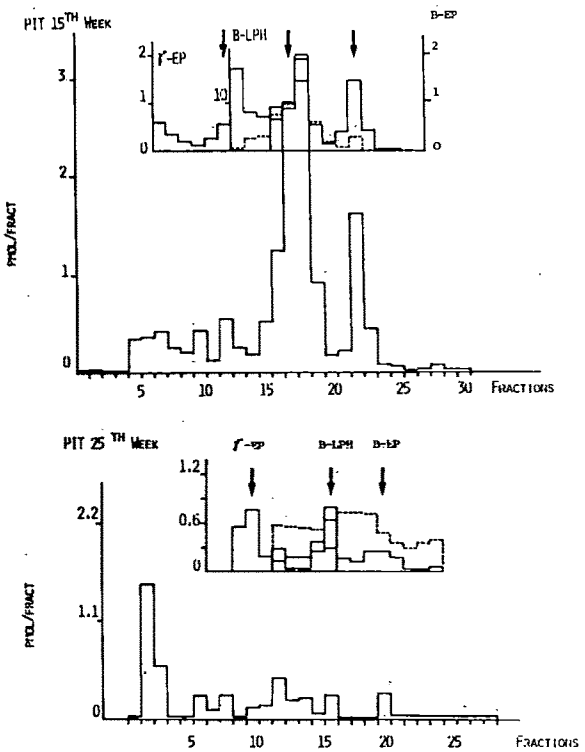


Fig. 2. Chromatogram of β-endorphin–like immunoreactivity and specific γ-endorphin *(γ-EP)*, β-lipotropin *(B-LPH)*, and β-endorphin *(B-EP)* immunoreactivity in two whole pituitaries obtained from a fetus at the fifteenth week and a fetus at the twenty-fifth week of pregnancy.

boiled for 10 minutes in 0.05 mol/L acetic acid in order to destroy proteases and then homogenized with a Polytron. After two successive centrifugations at 4000 × g for 15 minutes, clear supernatants were stored at −20° C until assayed. The pellets were treated with 1 mol/L sodium hydroxyde and submitted to a protein content evaluation with the use of the Folin phenol reagent.

High-performance liquid chromatography. Homogenates were injected in a high-performance liquid chromatography apparatus (Waters, Milford, Massachusetts) consisting of two pumps (Model 510), an ultraviolet detector (Model 440), and a gradient controller (Model 680). A reverse phase C-18 column (μBondapack, 39 by 300 mm, 10 μm size) was eluted in a linear gradient from 25% to 40% acetonitrile in 0.01 mol/L hydrochloric acid, for 15 minutes, at a flow rate of 1.5 ml/min. For each sample, 30 fractions were collected (each for 30 seconds), evaporated to dryness, and redissolved in 1 ml of 0.12 mol/L phosphate buffer, pH 7.4, and 0.1% bovine serum albumin.

In order to evaluate the amount of material sticking on the column, 1 ng of each of the three peptides was injected into the system and processed as described above. This experiment was replicated five times. Recoveries were 88.1% ± 10.4% for β-endorphin, 91.5% ± 17.3% for β-lipotropin, and 79.4% ± 12.8% for γ-endorphin.

Radioimmunoassays. Each fraction was tested for its β-endorphin–like immunoreactivity with the use of the anti–β-endorphin serum B4 (kindly donated by Dr. V. M. Wiegant, Utrecht, The Netherlands), which cross reacts with many β-endorphin–related peptides including 1-16 β-endorphin (260% on a molar basis), 1-17 β-endorphin (450%), 1-26 β-endorphin (11.5%), 1-27 β-endorphin (9.5%), and acetyl β-endorphin (100%). Thus, according to this β-endorphin immunoreactivity and in view of the retention times (Table I) of γ-endorphin, β-lipotropin, and β-endorphin, fractions were retested for their immunoreactivity with the use of appropriate iodinated peptides and specific antisera kindly donated by Dr. V. M. Wiegant (γ-endorphin) and by Professor C. H. Li (β-endorphin and β-lipotropin, San Francisco, California). Details of the different radioimmunoassays were previously reported.[2, 11]

Results

In the embryo of 4.7 weeks' gestation neither β-endorphin–like immunoreactivity nor specific peptides were detected. β-Endorphin (195.9 ± 57.7 fmol, mean ± SE) and β-lipotropin (569.6 ± 64.9 fmol)

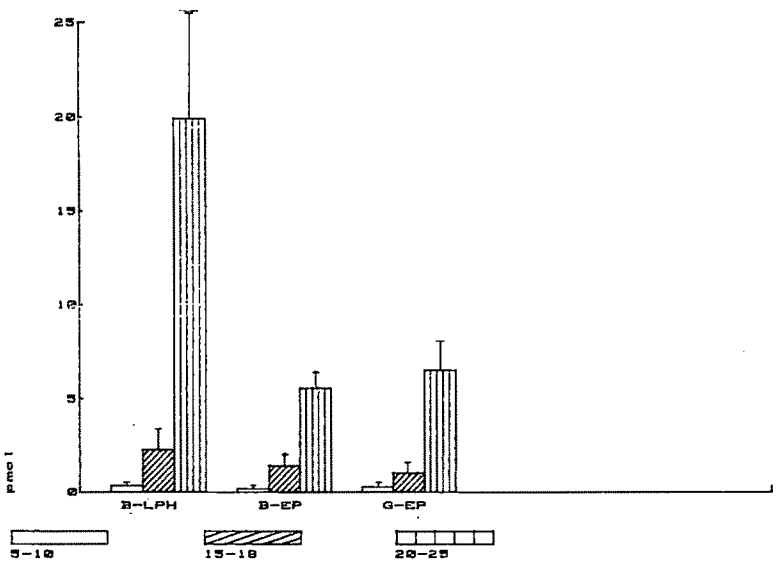


Fig. 3. Total content (mean ± SD) of β-endorphin *(B-EP)*, γ-endorphin *(G-EP)*, and β-lipotropin *(B-LPH)* in embryos and fetal pituitaries from 5 to 25 weeks of pregnancy.

were found but no γ-endorphin was present in embryos until the tenth week of pregnancy with one exception. This latter sample was the only one showing a poor β-lipotropin content.

During the embryonic period the peptides were measured only in the cephalic portion and no immunoreactivity was found in the caudal one (Fig. 1).

High amounts of the three proopiomelanocortin-related peptides were present in the fetal pituitaries; immunoreactive β-lipotropin, β-endorphin, and γ-endorphin elute at the same retention times as standard molecules (Fig. 2).

The total content of β-endorphin, β-lipotropin, and γ-endorphin in embryos and fetal pituitaries is shown in Fig. 3. There is a progressive increase of the three peptides throughout pregnancy, but the rates of such increases are different. This is reflected by the absence of correlation between β-lipotropin and β-endorphin, β-lipotropin and γ-endorphin, and γ-endorphin and β-endorphin during pituitary development.

Interestingly enough, the β-lipotropin/β-endorphin ratio increases from 1.41 ± 0.37 at the beginning of the second trimester to 4.4 ± 1.40 ($p < 0.5$) in the pituitaries of 22 to 25 weeks' gestational age. The opposite occurs in the β-endorphin/γ-endorphin ratio, which decreases from 1.64 ± 0.58 to 1.08 ± 0.29. In fetal pituitary extracts (Fig. 2), no β-endorphin–like immunoreactivity (lower part of each panel) was detected in fractions 24 to 30, corresponding to the elution pattern of acetyl β-endorphin.

Table I. Retention times, in minutes (mean ± SD of at least 10 determinations) with the use of a μBondapack C-18 RP column eluted from 25% to 40% acetonitrile in 0.01 mol/L hydrochloric acid, in 15 minutes at a flow rate of 1.5 ml/min

Peptide	*Retention time (min, mean ± SD)*
1-16 Endorphin	3.12 ± 0.07
1-17 Endorphin	6.5 ± 0.14
β-Lipotropin	9.4 ± 0.48
β-Endorphin	11.9 ± 0.69
Acetyl 1-31 endorphin	13.6 ± 0.58
1-26 Endorphin	14.4 ± 0.34
1-27 Endorphin	14.8 ± 0.48
Adrenocorticotropin 4-10	2.9 ± 0.31
α-Melanocyte–stimulating hormone*	3.62 ± 0.1
Adrenocorticotropin 1-39	8.1 ± 0.13
Met-enkephalin	3.6 ± 0.0

*Acetyl 1-13 adrenocorticotropin.

Comment

These data provide evidence that β-endorphin, β-lipotropin, and γ-endorphin are present in the fetal pituitary throughout development. Time-related differences in the appearance of the three peptides in the pituitary exist. β-Endorphin and β-lipotropin appeared in the seventh week when they were present in the cephalic portion, indicating that at that moment proopiomelanocortin activity was confined to pituitary and central nervous system structures.

Few studies exist about the ontogeny of β-lipotropin and β-endorphin in the human embryo. Some authors reported the presence of β-endorphin[6] and β-lipotropin[7] in the pituitary in the eleventh week of pregnancy when bioassayable adrenocorticotropic hormone is also demonstrable.[11] Immunohistochemical data have indicated positive staining for β-endorphin early in development, that is, in the seventh week,[4] and the present

biochemical characterization agrees completely with such a study.

γ-Endorphin, a product of β-endorphin cleavage that is present in brain synaptosomal membranes and in the adult pituitary,[14] is undetectable in all but one embryo, thus suggesting that the proopiomelanocortin-enzymatic system needs further maturational processes in intrauterine life in order to be fully expressed.

It was assumed that during fetal life pituitary proopiomelanocortin undergoes two successive cleavage steps: the first one to produce adrenocorticotropic hormone and β-lipotropin and the second one further cleaving these two peptides to give α-melanocyte–stimulating hormone and β-endorphin, respectively.[8] The second step of proopiomelanocortin processing is typical of the intermediate lobe of the rat pituitary,[13] a structure lacking in the human adult pituitary but well defined during fetal life. However, in whole fetal pituitaries and embryos, β-lipotropin was the predominant peptide and the β-lipotropin/β-endorphin ratio increased until the end of the second trimester, suggesting that the first proopiomelanocortin cleavage step is the most active in this period of life.

Gibbs et al.[14] superfused the fetal pituitary and demonstrated that the anterior lobe acquires its responsiveness to corticotropin-releasing factor just after the twentieth week of pregnancy. On the other hand, the neurointermediate lobe releases β-endorphin only after a potassium chloride–induced cell depolarization, and no changes occurred with corticotropin-releasing factor or dopamine. Thus the major peak of β-lipotropin obtained from whole pituitaries until the end of the second trimester can be interpreted as originating from the anterior lobe. We suggest that as early as the embryo the enzyme pattern for proopiomelanocortin cleavage is similar to that existing in the adult pituitary. Other authors,[15] studying human adrenocorticotropic hormone ontogeny, also concluded that the anterior lobe matures before the intermediate one. Another feature of the neurointermediate lobe (in the rat) is the acetylation of proopiomelanocortin and its related peptides, a process that inactivates the ability to bind opiate receptors.[16] It is uncertain if acetylation occurs in the human being and we found little immunoreactivity, if any, related to acetyl β-endorphin.

In conclusion proopiomelanocortin-related peptides are evident early in development and both β-endorphin and β-lipotropin were present in embryonic life, between 5 and 7 weeks, while smaller fragments of β-endorphin, such as γ-endorphin, have a delayed appearance. By the beginning of the second trimester the pituitary processing of proopiomelanocortin is similar to that of the adult and the functional activity of the anterior lobe seems to prevail over that of the "fetus-related" neurointermediate lobe around the twenty-fifth week of pregnancy.

We are indebted to Dr. V. Wiegant and Dr. P. Burbach of the Rudolf Magnus Institute for Pharmacology, University of Utrecht, The Netherlands, for their advice about high-performance liquid chromatography techniques and for the supply of the quoted materials. We also acknowledge Prof. A. E. Panerai, of the Istituto di Farmacologia, Università di Milano, for his help in performing our first high-performance liquid chromatography runs. Finally, the generous supply of anti–β-endorphin and anti–β-lipotropin sera from Prof. C. H. Li, of the Hormone Research Laboratory, University of California, San Francisco, is also acknowledged.

REFERENCES

1. Genazzani AR, Facchinetti F, Parrini D. Beta-lipotropin and beta-endorphin plasma levels during pregnancy. Clin Endocrinol 1981;14:409.
2. Baraldi M, Giarrè G, Santi M, Facchinetti F, Petraglia F, Genazzani AR. Pregnancy-related changes of opiate receptors identified in rat uterine membranes by ^{3}H naloxone binding. Peptides 1985;6:971.
3. Liotta A, Krieger DT. Biosynthetic pathway of ACTH and related peptides of the human placenta: in vitro demonstration. In: Klopper A, Genazzani AR, Crosignani PG, eds. Human placenta—proteins and hormones. London: Academic Press, 1980:267.
4. Dubois P, Vargues-Regairaz H, Dubois MP. Human foetal anterior pituitary immunofluorescent evidence for corticotropin and melanotropin activities. Zellforschung 1973; 45:131.
5. Begeot M, Dubois MP. Immunologic localization of alpha- and beta-lipotropin in corticotropic cells of the normal and anencephalic fetal pituitaries. Cell Tissue Res 1978; 193:413.
6. Holland D, Silman RE, Chard T, et al. Corticotropin and lipotropin "family tree" of the human pituitary gland. J Endocrinol 1979;83:488.
7. Brubaker PL, Baird AC, Bennett HPJ, Browne CA, Solomon S. Corticotropic peptides in the human fetal pituitary. Endocrinology 1982;111:1150.
8. Silman RE, Chard T, Lowry PJ, Smith I, Young IM. Human foetal pituitary peptides and parturition. Nature 1976;260:716.
9. Tilders FJH, Parker CR, Barnea A, Porter JC. The major immunoreactive α-melanocyte-stimulating hormone (α-MSH)-like substance found in human fetal pituitary tissue is not α-MSH but may be desacetyl α-MSH (adrenocorticotropin 1-13 NH2). J Clin Endocrinol Metab 1981;52:319.
10. Verfoef J, Wiegant VM, De Wied D. Regional distribution of alpha- and gamma-type endorphins in rat brain. Brain Res 1982;231:454.
11. Kaplan SL, Grumbach MM, Aubert ML. The ontogenesis of pituitary hormones and hypothalamic factors in the human fetus: maturation of central nervous system regulation of anterior pituitary function. Recent Prog Horm Res 1976;32:161.
12. Burbach JPH, Wiegant VM. Isolation and characterization of α-endorphin and γ-endorphin from single human pituitary glands. FEBS Lett 1984;166:267.
13. Przewlocki R, Hollt V, Voigt KH, Herz A. Distinctive

in vitro release of β-endorphin from the anterior compared to the posterior/intermediate lobe of rat pituitary. Life Sci 1979;24:1601.
14. Gibbs DM, Stewart RD, Liu JH, Vale W, Rivier J, Yen SSC. Effects of synthetic corticotropin-releasing factor and dopamine on the release of immunoreactive β-endorphin/β-lipotropin and α-melanocyte-stimulating hormone from human fetal pituitaries in vitro. J Clin Endocrinol Metab 1982;55:1149.
15. Allen RG, Pintar JE, Stack J, Kendall JW. Biosynthesis and processing of pro-opiomelanocortin-derived peptides during fetal pituitary development. Dev Biol 1985; 102:43.
16. Smyth DG, Massey DE, Zakarian S, Finnie MDA. Endorphins are stored in biologically active and inactive form: isolation of α-N-acetyl peptides. Nature 1979;279:252.

Effects of maternal anemia on cardiac output, systemic oxygen consumption, and regional blood flow in pregnant sheep

Daniel I. Edelstone, M.D., Mary E. Paulone, M.D., John J. Maljovec, M.D., and Margaret Hagberg, M.D.
Pittsburgh, Pennsylvania

We measured regional blood flows and cardiac output and calculated regional oxygen deliveries (blood flow × arterial blood oxygen concentration) and systemic oxygen delivery, oxygen extraction, and oxygen consumption as maternal hematocrit was varied randomly between 31% and 8% by exchange transfusion with plasma or packed sheep red blood cells. We observed three patterns of response: (1) In the heart, brain, and adrenal glands, oxygen delivery was constant or nearly constant over the range of hematocrits studied; (2) in the kidneys, splanchnic organs, and uteroplacental tissues, oxygen delivery was a linear function of hematocrit; (3) in the carcass tissues, oxygen delivery decreased slowly as hematocrits were reduced from 31% to 15% and dropped rapidly at hematocrits ≤15%. Systemic oxygen consumption was maintained at normal levels, despite reductions in hematocrit to about 15%, because oxygen extraction increased as compensation. At lower hematocrits, systemic oxygen consumption fell in association with a metabolic acidemia, suggesting that, with this severity of acute anemia, oxygen supply to some tissues in the pregnant sheep was inadequate for tissue metabolic demands. (Am J Obstet Gynecol 1987;156: 740-8.)

Key words: Acute isovolemic anemia, cardiac output distribution, maternal acid-base balance

At all developmental ages, anemia affects a wide variety of cardiovascular functions.[1-3] Two important functions affected are tissue blood flow and tissue oxygen delivery.[3,4] In nonpregnant animals, responses of individual organs and tissues to anemia vary between tissues, chiefly because of two factors: (1) Tissues regulate blood flow in direct response to changes in blood oxygen concentration or tissue oxygen availability; (2) anemia induces alterations in blood viscosity that directly influence blood flow.[3-5] Although alterations in viscosity (at any given shear rate) affect all organs and tissues equally, changes in vascular tone secondary to reductions in oxygen concentration or oxygen availability differ considerably among tissues. As such, the vascular effects of anemia show regional variations. Experiments done on fetal,[1] neonatal,[2] and nonpregnant adult animals[3] have shown that blood flows to the brain, heart, and adrenal glands increase sufficiently during anemia so that oxygen delivery (blood flow × arterial blood oxygen concentration) to these organs is maintained at normal or nearly normal levels. In contrast, in virtually all other organs, perfusion increases only modestly or not at all with anemia, and thus oxygen delivery falls as a function of decreasing hematocrit. Similar experiments have not been performed in pregnant animals. Such experiments would be of importance for at least two reasons. First, normal pregnancy is associated with considerable cardiovascular changes.[6] The ways in which maternal tissues respond to the further cardiovascular stresses of anemia may differ from those of the nonpregnant animal. Second, maternal anemia is a common complication of human pregnancy[7] and has been associated with increases in maternal morbidity and mortality rates. Data on the cardiovascular adjustments of pregnant animals to anemia may help to explain why morbidity and mortality rates are increased during pregnancy.

The purpose of our experiments was to identify the relationships of organ blood flows and oxygen deliv-

From the Department of Obstetrics and Gynecology, University of Pittsburgh School of Medicine, Magee-Womens Hospital.
This work was supported by grants from the National Institutes of Health (HD 16368) and from the Magee-Womens Hospital Research Fund.
This work was presented in part at the Thirtieth Annual Meeting of the Society for Gynecologic Investigation, Washington, D. C., March 17-20, 1983, and at the annual meeting of the American Physiological Society, Niagara Falls, New York, October, 1985. An abstract appeared in Physiologist 1985;28:352.
Received for publication July 17, 1986; revised September 22, 1986; October 10, 1986.
Reprint requests: Daniel I. Edelstone, M.D., Department of Obstetrics and Gynecology, Magee-Womens Hospital, Pittsburgh, PA 15213.

eries to maternal hematocrit during acutely induced anemia in pregnant animals. We also planned to measure cardiac output and systemic oxygen delivery and oxygen consumption during anemia to establish how the anemic state affects maternal oxidative metabolism. The fetal and placental responses to acute maternal anemia have previously been evaluated and are the subject of a separate report.[8]

Methods

Preparation of animals. We surgically prepared seven singleton pregnant sheep in which gestational age ranged from 105 to 125 days (term = 147 days). A spinal or epidural anesthetic (tetracaine hydrochloride, 1%) was administered to each sheep, after which catheters were inserted into two maternal hind limb arteries and one vein. Catheters were passed in a retrograde direction via a carotid artery into the left ventricle and via a jugular vein into the right ventricle. Catheter placements were verified by observation of the characteristic pressure wave forms produced by the left and right ventricles. Two to 4 days later, we studied the unanesthetized mothers as they stood quietly in an open transport cage. Five of the seven animals were studied on the fourth postoperative day, one on the third, and one on the second.

Experimental procedures. We measured cardiac output, systemic oxygen delivery and oxygen consumption, acid-base balance, and regional blood flows and oxygen deliveries at maternal hematocrits ranging from 31% to 8%. To vary the maternal hematocrit, we performed isovolemic exchange transfusions by infusing packed sheep red blood cells or plasma through one vascular catheter while withdrawing blood at the same rate from a second catheter.[1-3] Hematocrits were changed gradually over a period of 1 to 4 hours, during which time arterial blood pressure and heart rate were continuously monitored (Statham P23Db transducers) and recorded (Beckman R611A polygraph recorder).

As the exchange transfusions were progressing, we measured hematocrit periodically. When hematocrit had reached a value selected randomly, we stopped the exchange transfusion. After maternal hematocrit, heart rate, and arterial blood pressure were stable for at least 30 minutes, we withdrew 0.6 ml blood samples simultaneously from the arterial and right ventricular catheters. We analyzed these samples within 15 minutes for the following: P_{O_2}, P_{CO_2}, and pH with a blood gas analyzer (Instruments Laboratories), oxygen concentration with an oxygen content analyzer (Lex-O_2-Con), and hematocrit with a microhematocrit centrifuge.[1] Immediately after the blood sampling, we injected radionuclide-labeled microspheres (labeled with either iodine 125, cerium 141, chromium 51, strontium 85, niobium 95, or scandium 46, 15 μm in diameter) into the left ventricle while withdrawing an arterial blood reference sample at a rate of 7.9 ml/min.[9] We used this high withdrawal rate during the microsphere injections to ensure that blood flow measurements made in the anemic animals were accurate.[10] The volume of maternal blood drawn during each study was replaced with whole blood or plasma so that a constant intravascular blood volume could be maintained.

In preliminary experiments we determined that we needed to inject 2 to 5 × 10^6 microspheres to have sufficient numbers of particles in the organs and in the reference arterial blood samples from which precise calculations of organ blood flows could be made. There were no cumulative effects of multiple injections of microspheres (maximum total spheres injected = 20 × 10^6) on maternal arterial blood pressure or heart rate.[9] In all cases arterial blood pressure and heart rate were stable before, during, and after the microsphere injections. When the measurements were completed at any given maternal hematocrit, we increased or decreased the hematocrit to a new level and repeated the oxygen and flow measurements. An average of five such measurements (range three to six) was made in each of the seven sheep. The exact hematocrit at which measurements were made varied from one sheep to the next. No attempt was made to study all animals at the same hematocrit levels.

Preparation of tissues and calculation and analysis of data. At the completion of the experiment, sheep were killed with an overdose of sodium pentobarbital, and catheter locations were verified visually. All organs were dissected, weighed, and processed further with standard techniques.[9] Organs were incinerated individually at 280° C for 48 hours, after which the ashed tissues were packed into counting vials. The amounts of each radionuclide in the organs and arterial blood reference samples were measured with a well-type gamma scintillation counter (Model 1185R, Tracor) and a 1024-channel pulse-height analyzer (Ultima II, Norland). Nuclide isotope separation was performed by computer, which used a least-squares fitting analysis to distinguish the various isotopes contained in each sample (Nova 3, Data General; average error in the calculation of corrected radioactive counts per minute for each isotope was <5%). Arterial blood reference samples and all organs, except the adrenal glands, contained >1500 microspheres to ensure that the error in any blood flow measurement was <5% at the 95% confidence limits.[9] For the adrenal glands all tissues contained >400 microspheres (maximum error 10%, 95% confidence limits).

We computed the organ blood flows and oxygen deliveries as follows[1-3]:

$$\text{Organ blood flow} = (CPM_{organ}/CPM_{ref}) \cdot \dot{Q}_{ref}$$
$$\text{Organ oxygen delivery} = (\text{organ blood flow}) \cdot CaO_2$$

where CPM_{organ} = radioactive counts per minute in the

Table I. Effects of acute maternal anemia on heart rate, mean arterial blood pressure, and arterial blood oxygen concentration and acid-base balance

	Hematocrit range				
	≥26%	*21%-25%*	*16%-20%*	*11%-15%*	*≤10%*
n	4	4	9	8	7
Heart rate (bpm)	96 ± 13	98 ± 10	113 ± 19	118 ± 10*	132 ± 12*
Mean arterial blood pressure (mm Hg)	94 ± 8	91 ± 8	86 ± 10	83 ± 16	85 ± 6
Arterial blood oxygen concentration (ml oxygen/dl blood)	12.6 ± 1.4	10.9 ± 1.3	8.8 ± 0.8*	6.8 ± 0.6*	4.5 ± 1.0*
Arterial blood					
pH	7.57 ± 0.01	7.62 ± 0.04	7.58 ± 0.04	7.58 ± 0.06	7.58 ± 0.02
P_{O_2} (mm Hg)	91 ± 2	95 ± 5	97 ± 8	98 ± 6	103 ± 9
P_{CO_2} (mm Hg)	29 ± 4	28 ± 4	29 ± 4	29 ± 5	24 ± 5*
Base excess (mEq/L)	6 ± 3	7 ± 6	5 ± 4	4 ± 2	−1 ± 3*

Values are means ± SD.
*$p < 0.05$, analysis of variance.

organ, CPM_{ref} = radioactive counts per minute in the arterial blood reference sample, $\dot{Q}ref$ = arterial reference blood flow, determined by calibration of the withdrawal pump, and CaO_2 = arterial blood oxygen concentration.

Cardiac output was calculated as follows[3,6]:

$$\text{Cardiac output} = (CPM_{total}/CPM_{ref}) \cdot \dot{Q}_{ref}$$

where CPM_{total} = total radioactive counts per minute injected into the left ventricle. To determine the total counts per minute injected, we used an aliquot system.[3] This method has been shown in other laboratories[3,6] to provide accurate measurements of cardiac output when proper precautions for counting high rates are taken. To be certain of its validity in our own laboratory, we calculated cardiac output as above and also as follows in two of the animals. In these two sheep all tissues were counted for radioactivity. Cardiac output was then equal to the sum of the blood flows to all of the individual organs plus the carcass tissues (skeletal muscle, skin, skeleton, and adipose tissue). Cardiac output calculated by the two methods differed by an average of 11% (±3% SD; n = 10 measurements made in two sheep). In the remaining five animals in which flow and oxygen delivery to carcass tissues were not measured directly, these variables were calculated as follows:

$$\text{Carcass blood flow} = \text{cardiac output} - \text{sum of all organ blood flows}$$

$$\text{Carcass oxygen delivery} = (\text{carcass blood flow}) \cdot CaO_2$$

Three variables of systemic oxygenation were computed with modifications of the Fick principle[5]:

$$\text{Systemic oxygen delivery} = (\text{cardiac output}) \cdot CaO_2$$

$$\text{Systemic oxygen consumption} = (\text{cardiac output}) \cdot (CaO_2 - C\bar{v}O_2)$$

$$\text{Systemic oxygen extraction} = (CaO_2 - C\bar{v}O_2)/CaO_2$$

where $C\bar{v}O_2$ = oxygen concentration in right ventricular blood.

There were no obvious diferences in basal maternal blood pressures, heart rates, or hematocrits as functions of gestational age (over the range studied, 109 to 127 days) or length of postoperative recovery period (2, 3, or 4 days). Therefore we based our analyses on the pooled data (32 measurements obtained in seven sheep). Two analyses were performed. First, organ blood flows and oxygen deliveries, maternal cardiac output, and systemic oxygen delivery, oxygen extraction, and oxygen consumption (dependent variables) were plotted against maternal hematocrit (independent variable). We analyzed the data by means of a weighted least-squares method based on covariance analysis that allowed us to generate regression lines that took into account responses seen in individual animals.[11] Linear, logarithmic, and power functions were all tested for best fit; the function selected as best describing each relationship had the greatest correlation coefficient associated with the least residual sum of squares. Second, we arbitrarily divided up the data into five equal hematocrit ranges so that we could present all of our data in tabular form. These data were analyzed with a one-way analysis of variance.[11] When significant differences were observed, we used the Newman-Keuls test (with Kramer's modification for comparisons involving unequal n) to identify which dependent variables differed from the rest.[11] In all statistical analyses, p values <0.05 were used to indicate statistically significant correlations.

Results

In the seven pregnant sheep studied, gestational age was 115 ± 7 days (mean ± SD) on the day of study, body weight was 42 ± 6 kg, and hematocrit was

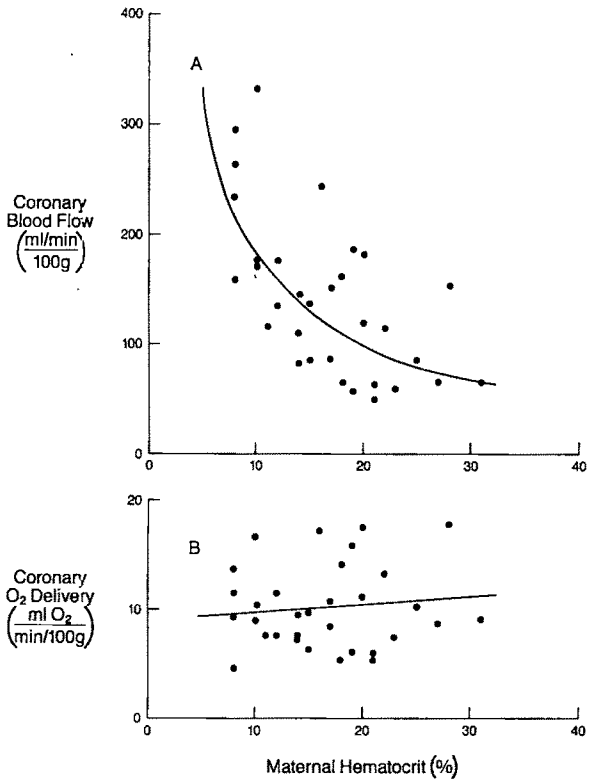


Fig. 1. Example of first pattern of response to acute maternal anemia. Coronary blood flow and oxygen delivery as functions of maternal hematocrit. *A*, Blood flow versus hematocrit, $y = 1365x^{-0.87}$; $r = 0.65$, $p < 0.001$. *B*, Oxygen delivery versus hematocrit, $y = 9.0 + 0.07x$; $r = 0.11$, NS.

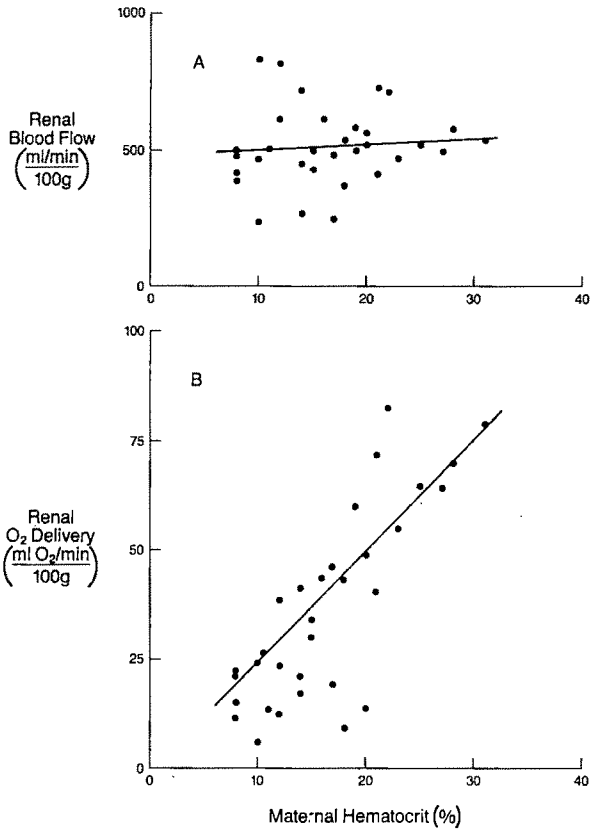


Fig. 2. Example of second pattern of response to acute maternal anemia. Renal blood flow and oxygen delivery as functions of maternal hematocrit. *A*, Blood flow versus hematocrit, $y = 467 + 2.3x$; $r = 0.10$, NS. *B*, Oxygen delivery versus hematocrit, $y = -0.22 + 2.56x$; $r = 0.81$, $p < 0.001$.

27% ± 2%. In response to reductions in maternal hematocrit, heart rate increased linearly, mean arterial blood pressure did not change significantly, arterial blood oxygen concentration fell linearly, and a combination of metabolic acidosis (decreased base excess) and respiratory alkalosis (decreased P_{CO_2}) developed (Table I).

Blood flow and oxygen delivery to maternal organs and tissues during acute isovolemic anemia could be classified into three general patterns of response. Figs. 1 to 3 illustrate the data obtained on three specific tissues, heart, kidneys, and carcass, that serve as examples of the three responses observed. Tables II and III present the data for all the organ and tissue responses, expressed in units of absolute flow and oxygen delivery (Table II) or as percentages of cardiac output (Table III).

In the first pattern, typified by the heart, brain, and adrenal glands, blood flow (absolute flow in Table II and flow as a percentage of total cardiac output in Table III) increased as maternal hematocrit decreased. In the case of the heart (Fig. 1, Table II), oxygen delivery was constant over the range of hematocrits studied. In the brain and adrenal glands, however, oxygen delivery decreased as maternal hematocrit was reduced (Table II). In the second pattern of response, organ blood flows did not change significantly in response to acute maternal anemia (Tables II and III). Because of these blood flow responses, organ oxygen deliveries decreased linearly as hematocrit was reduced. Organs exhibiting this second response pattern included the kidneys, stomach, intestine, colon, spleen, liver, and uteroplacenta. Fig. 2 illustrates the data for the renal response to acute maternal anemia as an example of this response pattern. The third pattern of response was observed in carcass tissues (skeletal muscles, skeleton, skin, and adipose tissues). Fig. 3 and Table II show that blood flow to carcass tissues increased as hematocrit was reduced to 15%, then decreased at lower hematocrit levels. Oxygen delivery did not change significantly as hematocrit was decreased from 31% to about 15% but fell rapidly when hematocrit was lowered below about 15%.

Because of the flow responses to acute maternal anemia observed in the various maternal organs and tissues, cardiac output increased as hematocrit was de-

Table II. Effects of acute maternal anemia on organ blood flows and oxygen deliveries

	Hematocrit range				
	≥26%	*21%-25%*	*16%-20%*	*11%-15%*	*≤10%*
n	4	4	9	8	7
Response pattern No. 1					
Heart					
Q	92 ± 50	74 ± 26	125 ± 51	131 ± 35	228 ± 65*
DO_2	11.6 ± 5.0	8.1 ± 3.1	11.1 ± 4.5	9.0 ± 3.3	10.5 ± 3.8
Brain					
Q	57 ± 8	62 ± 17	80 ± 32	79 ± 18	101 ± 44*
DO_2	7.2 ± 0.6	6.8 ± 2.4	7.0 ± 1.4	5.4 ± 1.4*	4.5 ± 2.2*
Adrenals					
Q	115 ± 23	122 ± 67	145 ± 50	162 ± 35*	217 ± 52*
DO_2	14.4 ± 2.6	13.3 ± 5.3	12.6 ± 3.9	11.0 ± 2.2	9.8 ± 3.6*
Response pattern No. 2					
Kidneys					
Q	528 ± 46	555 ± 140	489 ± 118	485 ± 185	474 ± 173
DO_2	66.2 ± 6.4	60.3 ± 18.6	43.1 ± 18.9	32.9 ± 10.9*	21.1 ± 7.4*
Stomach					
Q	19 ± 30	17 ± 17	15 ± 19	17 ± 13	24 ± 6
DO_2	2.6 ± 4.3	1.8 ± 2.3	1.3 ± 1.3	1.1 ± 0.8	1.0 ± 0.4*
Small intestine					
Q	72 ± 19	62 ± 12	61 ± 13	76 ± 17	82 ± 10
DO_2	9.1 ± 2.0	6.8 ± 3.8	5.3 ± 1.0*	5.2 ± 1.1*	3.7 ± 0.7*
Colon					
Q	51 ± 16	43 ± 19	46 ± 22	42 ± 15	42 ± 7
DO_2	6.6 ± 1.7	4.7 ± 2.2	4.1 ± 1.9	3.0 ± 1.2*	1.8 ± 0.6*
Spleen					
Q	175 ± 104	191 ± 145	172 ± 101	147 ± 52	135 ± 56
DO_2	22.0 ± 12.9	20.8 ± 15.5	15.0 ± 8.6	10.0 ± 4.6	6.0 ± 2.1*
Liver					
Q	152 ± 126	138 ± 98	151 ± 77	156 ± 62	173 ± 50
Uterus plus placenta					
Q	329 ± 108	304 ± 92	304 ± 120	269 ± 40	272 ± 42
DO_2	41.5 ± 9.2	33.1 ± 12.4	26.8 ± 12.6	18.3 ± 2.2*	12.3 ± 3.6*
Response pattern No. 3					
Carcass					
Q	6 ± 2	6 ± 2	8 ± 2*	6 ± 1	3 ± 1*
DO_2	0.7 ± 0.2	0.6 ± 0.2	0.7 ± 0.2	0.4 ± 0.1*	0.1 ± 0.1*

Values are means ± SD. Q = blood flow; DO_2 = oxygen delivery; both are expressed as milliliters per minute per 100 gm, except for uterus plus placenta, expressed per kilogram of uterus plus placenta plus fetus. Note that liver blood flow is the sum of hepatic arterial and portal venous blood flows; portal blood flow is the sum of the arterial blood flows to all gastrointestinal tract organs. Without data on portal venous blood oxygen concentration, total liver oxygen delivery could not be calculated.

*$p < 0.05$, analysis of variance.

creased to 15% and then began to decrease when maternal hematocrit was reduced below about 15% (Table III and Fig. 4, *A*). Systemic oxygen delivery decreased gradually as hematocrit was reduced from 31% to 15% but fell more rapidly at lower hematocrits (Fig. 4, *B*). As compensation for the fall in systemic oxygen delivery, oxygen extraction increased (Fig. 5, *A*) so that systemic oxygen consumption could be maintained over the range of maternal hematocrits from 31% to about 15% (Fig. 5, *B*). At hematocrits ≤15%, systemic oxygen consumption began to decrease. The decrease in oxygen consumption at hematocrits ≤10% was associated with a combined metabolic acidemia (reduced arterial blood base excess) and a respiratory alkalemia (reduced arterial blood P_{CO_2}) (Table I).

Comment

Oxygen delivery to tissues is a function of two variables, the tissue blood flow and the arterial blood oxygen concentration.[1-4] At normal hematocrits blood flow is primarily determined by the cross-sectional area of the vasculature and by the perfusion pressure.[3] When hematocrit is altered, however, the attendant changes in blood viscosity also directly influence tissue perfusion. Because the relative contributions of vascular geometry and viscosity to blood flow regulation differ from one vascular bed to another,[3-5] the effect of anemia on blood flow and oxygen delivery to tissues shows regional variations. In our experiments on pregnant sheep, we identified three general patterns of response of regional blood flows and oxygen deliveries as func-

Table III. Effects of acute maternal anemia on distribution of cardiac output

	Hematocrit range				
	≥26%	*21%-25%*	*16%-20%*	*11%-15%*	*≤10%*
n	4	4	9	8	7
Cardiac output distribution					
Cardiac output (ml/min/kg)	111 ± 3	120 ± 17	130 ± 11*	113 ± 24	102 ± 10
Response pattern No. 1					
Heart	3.9 ± 1.4	3.0 ± 0.8	4.6 ± 2.1	6.4 ± 1.0*	9.5 ± 3.0*
Brains	1.2 ± 0.2	1.3 ± 0.6	1.6 ± 0.5	2.0 ± 1.6	3.0 ± 1.6*
Adrenals	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1*
Response pattern No. 2					
Kidneys	12.0 ± 2.0	11.9 ± 4.0	10.0 ± 2.9	11.5 ± 3.4	12.3 ± 4.1
Stomach	5.7 ± 2.8	4.2 ± 4.6	4.2 ± 5.6	5.2 ± 4.1	6.7 ± 4.3
Small intestine	8.2 ± 4.2	6.7 ± 2.8	7.6 ± 3.6	8.1 ± 3.7	9.3 ± 2.6
Colon	7.0 ± 3.4	6.5 ± 3.5	6.0 ± 2.8	6.0 ± 2.1	7.7 ± 1.4
Spleen	3.2 ± 1.4	3.3 ± 1.7	2.7 ± 1.5	2.5 ± 1.2	2.5 ± 0.9
Liver	24.6 ± 4.0	21.3 ± 4.2	21.2 ± 2.9	23.1 ± 4.6	26.8 ± 4.8
Uterus plus placenta	13.7 ± 4.2	15.8 ± 5.2	13.6 ± 3.4	14.7 ± 4.6	16.5 ± 4.5
Response pattern No. 3					
Carcass	44.6 ± 7.3	46.5 ± 8.2	48.9 ± 8.7	42.2 ± 8.5	31.6 ± 6.4*

Values are means ± SD. Data for cardiac output distribution are percentages of the total cardiac output.
*$P < 0.05$, analysis of variance.

tions of maternal hematocrit during acutely induced anemia.

In the first pattern of response, seen in the heart, brain, and adrenal glands, blood flow varied inversely with hematocrit so that oxygen delivery to these organs was held constant or decreased modestly over about a fourfold range of hematocrits (8% to 31%) and a fourfold range of arterial blood oxygen concentrations (3.9 to 14.6 ml of oxygen per deciliter of blood). A portion of the increase in blood flow was probably due to the rheologic effects of reduced blood viscosity that occur during anemia.[4, 5] This increase by itself, however, would not account fully for the large increases in organ perfusion that we observed.[3, 5] Since perfusion pressure did not change during acutely induced anemia, some of the rise in blood flow in the heart, brain, and adrenals must have been due to vasodilation. In the heart of the pregnant sheep, the combination of decreased viscosity and coronary vasodilation produced sufficient increases in perfusion for coronary oxygen delivery to be unaffected by the degrees of acute maternal anemia produced. In contrast, in the brain and adrenal glands, blood flow increased to a lesser extent than that required for maintenance of oxygen delivery. Thus oxygen delivery decreased slightly as maternal hematocrit was reduced. Our observations on the responses of the heart, brain, and adrenals to acute anemia in pregnant animals are similar to those made in nonpregnant adult animals[3] and in fetal[1] and neonatal[2] animals, indicating that the coronary, cerebral, and adrenal circulatory responses to anemia are qualitatively the same at all stages of development.

In the second pattern of response to acute maternal anemia, blood flow showed no net effect as hematocrit was reduced. Since the effects of viscosity, at any given shear rate, are uniform throughout all vascular beds,[3-5] perfusion should have increased as viscosity was reduced. The lack of an increase in blood flow to the kidneys, the splanchnic organs, and the uterus-placenta suggests that these circulations were vasoconstricted during maternal anemia. This latter interpretation may appear to be unsupported by our data, since we showed that vascular resistance, calculated as mean arterial blood pressure divided by blood flow, did not change in these organs. However, a constant resistance indicates the lack of either vasoconstriction or vasodilation only when hematocrit and thus viscosity are also constant.[3] When hematocrit and viscosity vary, vascular resistance must be considered to be primarily a function of two components,[3] the blood viscosity and the cross-sectional area of the vasculature, as follows: $R = 8\eta l/\pi r^4$, where R = resistance, η = viscosity, l = length of the vascular channel, and r = radius of the vascular channel (a measure of the cross-sectional area). A decrease in viscosity, η, due to anemia would be expected to lead to a reduction in resistance, R. Our data, however, indicated no such reduction. Therefore the radius, r, of the vascular channel must also have decreased as a result of vasoconstriction (the length of the vascular channel, l, is fixed anatomically and would not be expected to vary during anemia).

The splanchnic, renal, and uterine vascular beds vasoconstrict in response to a variety of stresses[12, 13] that are associated with sympathetic nerve stimulation or

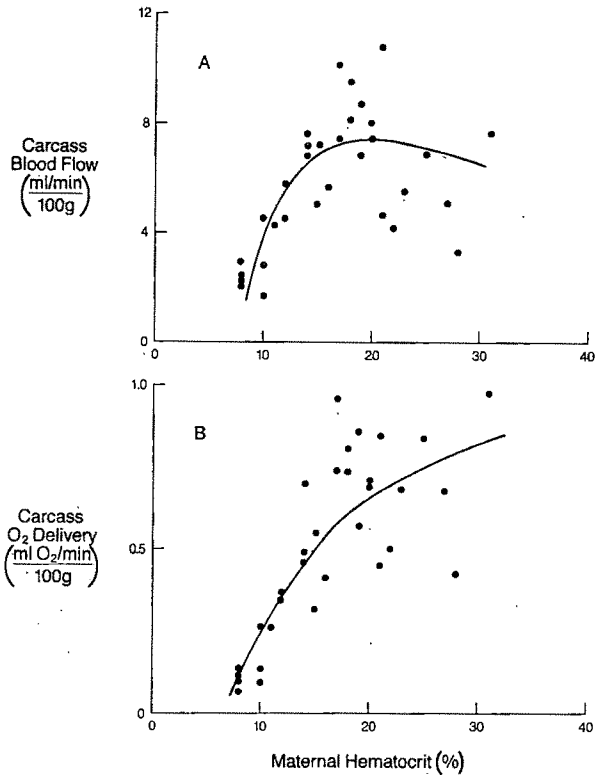


Fig. 3. Third pattern of response to acute maternal anemia. Relationship of blood flow and oxygen delivery to carcass tissues (skeletal muscle, skin, skeleton, and adipose tissues) to maternal hematocrit. *A*, Blood flow versus hematocrit, $y = (157 \ln x - 324)/x$; $r = 0.65$, $p < 0.001$. *B*, Oxygen delivery versus hematocrit, $y = -1.06 + 0.57 \ln x$; $r = 0.81$, $p < 0.001$.

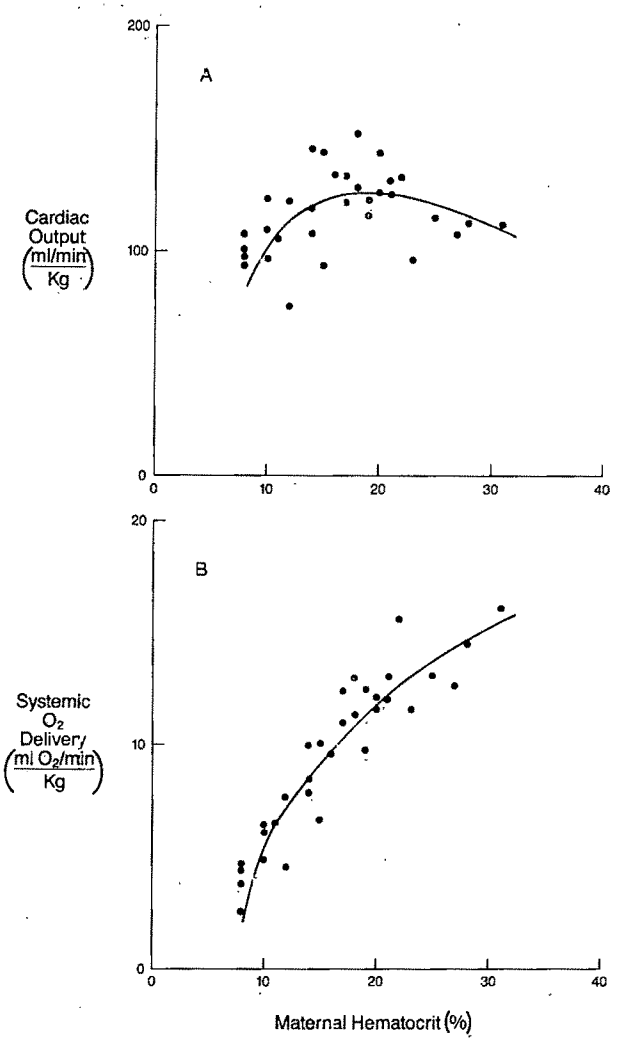


Fig. 4. Cardiac output and systemic oxygen delivery (both per kilogram of sheep) as functions of maternal hematocrit. *A*, Cardiac output versus hematocrit, $y = (2204 \ln x - 4125)/x$; $r = 0.70$, $p < 0.001$. *B*, Oxygen delivery versus hematocrit, $y = -14.7 + 8.86 \ln x$; $r = 0.94$, $p < 0.001$.

catecholamine release. Acute anemia may also produce vasoconstriction that is mediated neurohumorally. Since perfusion to the kidneys, splanchnic organs, and uterus did not vary during maternal anemia, oxygen delivery to these organs was directly dependent on hematocrit. Despite such a dependence, the quantity of oxygen utilized by these organs must have been maintained as hematocrits were lowered to about 15%, since systemic and uteroplacental oxygen consumptions[8] were unaffected by decreases in maternal hematocrit to similar levels. At hematocrits ≤10%, the oxygen supply to these tissues must have been inadequate for aerobic metabolism, judging by the reduction in systemic and uteroplacental[8] oxygen consumptions that developed.

In the third pattern of response to maternal anemia, seen in the carcass tissues, blood flow increased as hematocrit was decreased to about 15% and then dropped rapidly as hematocrit was reduced to lower levels. The initial rise in carcass perfusion could be accounted for entirely by decreases in blood viscosity,[5] in the absence of any active vasoregulation. However, when hematocrits were reduced to less than about 15%, the decrease in perfusion we observed must have been due to substantial degrees of vasoconstriction in carcass tissues, which outweighed the beneficial effects of further reductions in viscosity on tissue perfusion.[3, 4] As with the kidneys, splanchnic organs, and uterus, vasoconstriction in carcass tissues was probably a result of enhanced sympathetic nerve activity or catecholamine secretion.[12] Although vasoconstriction was present to some extent in organs and tissues exhibiting both the second and third patterns of response, its effects were more pronounced in the tissues of the carcass than in those of the abdominal viscera.

The changes we observed in cardiac output and systemic oxygen delivery as functions of maternal hematocrit were the summation of all the individual organ

and tissue responses to maternal anemia. Cardiac output increased as hematocrit was reduced to about 15%, because blood flow to the heart, brain, adrenal glands, and carcass tissues increased. Cefalo et al.,[14] however, found that isovolemic anemia, when initiated rapidly (<1 hour), did not produce an increase in cardiac output in anesthetized sheep, even though systemic vascular resistance fell. Differences in experimental design between their study and ours may explain the differing responses of cardiac output to acute anemia. In our experiments, when hematocrits were dropped below about 15%, cardiac output fell to values seen when hematocrits were ≥26%, because the continued increases in perfusion to the heart, brain, and adrenals were offset by progressive decreases in carcass blood flow.

Although cardiac output increased during reductions in hematocrit of up to 50% below normal levels, the increases were less than the associated decreases in arterial blood oxygen concentration, and thus systemic oxygen delivery fell. Despite the fall in total oxygen delivery, systemic oxygen consumption was unaffected by acute variations in hematocrit over the range of hematocrits from 31% to about 15%, because systemic oxygen extraction increased sufficiently as compensation. Similar responses have been observed in nonpregnant adult animals.[15] At lower hematocrits, systemic oxygen consumption began to decline in association with the development of a metabolic acidemia (decrease in arterial blood base excess) and a respiratory alkalemia (decrease in arterial blood P_{CO_2}). The metabolic acidemia suggests that oxygen delivery to some tissues, most likely those of the carcass, was insufficient for oxidative metabolic demands. The respiratory alkalemia was due to hyperventilation that could have resulted from three possible mechanisms[16]: (1) Lactacidemia resulting from anaerobic metabolism is a potent stimulus for ventilation; (2) anemia directly increases chemoreceptor activity and augments respiration; (3) increased sympathetic nerve activity stimulates chemoreceptors, which in turn enhance ventilation.

The level of maternal hematocrit associated with a fall in systemic oxygen consumption and the development of a metabolic acidemia (anaerobic metabolism) was about the same as that which led to anaerobic metabolism in the fetus.[8] This observation might suggest that the maternal organism is not necessarily protected from the adverse effects of anemia at the expense of the well-being of the conceptus. However, if fetal anaerobic metabolism were to develop during severe degrees of maternal anemia and if the anemia were not corrected promptly, fetal death would ensue. After the fetus died, uteroplacental perfusion would fall. The portion of maternal cardiac output designated for uteroplacental tissues would then be available to other maternal tissues whose perfusions and oxygen supplies might have been inadequate for oxidative metabolism. In this way a severely anemic mother could survive, even if her fetus could not. Our current data cannot and should not be extrapolated to encompass chronic degrees of anemia in animal or human pregnancies. It is unknown how chronic anemia might affect the maternal systemic circulation, although in nonpregnant animals acute anemia and chronic anemia produce similar adjustments in cardiac output and oxidative metabolic rate.[17]

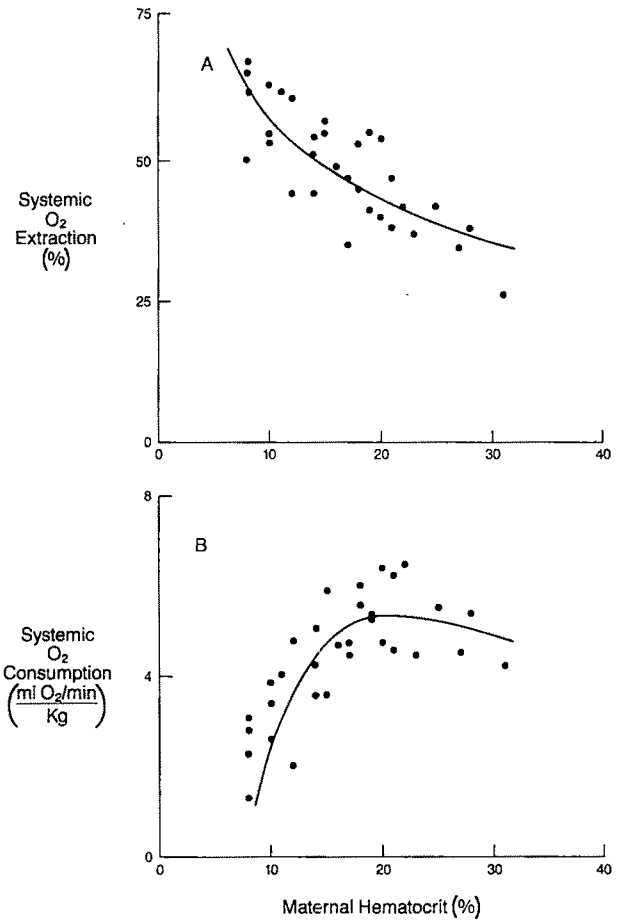


Fig. 5. Systemic oxygen extraction and oxygen consumption (per kilogram of sheep) as functions of maternal hematocrit. *A*, Oxygen extraction versus hematocrit, $y = 159x^{-0.44}$; $r = 0.82$, $p < 0.001$. *B*, Oxygen consumption versus hematocrit, $y = (114 \ln x - 236)/x$; $r = 0.83$, $p < 0.001$.

In summary our data indicate that the organs and tissues of the pregnant sheep show regional variations in their responses to acute isovolemic anemia. Blood flows to the heart, brain, and adrenal glands increase as compensation for reductions in hematocrit. Oxygen delivery to those organs is affected little by reductions in hematocrit and thus in arterial blood oxygen con-

centration. In all other organs and tissues, blood flows either are affected modestly or are reduced during anemia, with the result that oxygen delivery to these latter tissues decreases as a function of maternal hematocrit. Despite reductions in oxygen delivery to many tissues, systemic oxidative metabolism can be sustained at normal levels even when hematocrit is reduced by as much as 50% below normal levels. The responses observed in pregnant animals are qualitatively similar to those noted for nonpregnant animals and for fetal and neonatal animals, indicating that the pattern of the cardiovascular response to acute anemia is the same throughout all stages of development.

We wish to thank Anthony Battelli, Pat Moran, and Lynn Heckman for their excellent technical assistance.

REFERENCES

1. Fumia FD, Edelstone DI, Holzman IR. Blood flow and oxygen delivery to fetal organs as functions of fetal hematocrit. Am J Obstet Gynecol 1984;150:274-82.
2. Holzman IR, Tabata B, Edelstone DI. Blood flow and oxygen delivery to the organs of the neonatal lamb as a function of hematocrit. Pediatr Res 1986;20:1274-9.
3. Fan F-C, Chen RYZ, Schuessler GB, Chien S. Effects of hematocrit variations on regional hemodynamics and oxygen transport in the dog. Am J Physiol 1980;238: H545-52.
4. Crowell JW, Smith EE. Determinant of the optimal hematocrit. J Appl Physiol 1967;22:501-4.
5. Nihill MR, McNamara DG, Vick RL. The effects of increased blood viscosity on pulmonary vascular resistance. Am Heart J 1976;92:65-72.
6. Rosenfeld CR. Distribution of cardiac output in ovine pregnancy. Am J Physiol 1977;232:H231-5.
7. Garn SM, Ridella SA, Petzold AS, Falkner F. Maternal hematologic levels and pregnancy outcomes. Semin Perinatol 1981;5:155-62.
8. Paulone ME, Edelstone DI, Shedd A. Effects of maternal anemia on uteroplacental and fetal oxidative metabolism in sheep. Am J Obstet Gynecol 1987;156:230-6.
9. Heymann MA, Payne BD, Hoffman JIE, Rudolph AM. Blood flow measurements with radionuclide-labeled particles. Prog Cardiovasc Dis 1977;20:55-79.
10. Rosenberg AA, Jones MD Jr, Koehler RC, Traystman RJ, Lister G. Precautions for measuring blood flow during anemia with the microsphere technique. Am J Physiol 1983;244:H308-11.
11. Zar JH. Biostatistical analysis. Englewood Cliffs, New Jersey Prentice-Hall, 1974.
12. Adachi H, Strauss HW, Ochi H, Wagner HN Jr. The effect of hypoxia on the regional distribution of cardiac output in the dog. Circ Res 1976;39:314.
13. Greiss FC Jr. A clinical concept of uterine blood flow during pregnancy. Obstet Gynecol 1967;30:595-604.
14. Cefalo RC, Seeds JW, Proctor HJ, Baker VV. Maternal and fetal effects of exchange transfusion with a red blood cell substitute. Am J Obstet Gynecol 1984;148:859-67.
15. Jan K-M, Chien S. Effect of hematocrit variations on coronary hemodynamics and oxygen utilization. Am J Physiol 1977;233:H106-13.
16. Berger AJ, Mitchell RA, Severinghaus JW. Regulation of respiration. N Engl J Med 1977;297:92-7, 138-43, 194-201.
17. Neill WA, Oxendine JM, Moore SC. Acute and chronic adjustments to induced anemia in dogs. Am J Physiol 1969;217:710-4.

Bound volumes available to subscribers

Bound volumes of the American Journal of Obstetrics and Gynecology are available to subscribers (only) for the 1987 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 156 (January-June) and Vol. 157 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the Journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular Journal subscription.

Myocardial adenylate cyclase activity in ritodrine-treated pregnant rabbits

Christos G. Hatjis, M.D., and J. D. Bottoms, B.S.
Winston-Salem, North Carolina

To determine whether prolonged exposure to ritodrine results in down-regulation of myocardial adenylate cyclase activity during pregnancy, New Zealand White rabbits with time-dated pregnancies were randomly assigned to a sham-operated control group and to a ritodrine-treated group. Starting at 22 days' gestation (term 31 days), animals received either intravenous ritodrine intermittently or the equivalent volume of vehicle for 3 days. Adenylate cyclase activity was determined in a plasma membrane fraction obtained from ventricular tissue. There was no difference in the basal activity or the basal and guanosine triphosphate activity between the two groups. L-Isoproterenol–dependent stimulation required the presence of guanosine triphosphate. Both maximal and 50% enzymatic stimulation were equivalent in both groups. Furthermore, sodium fluoride–dependent stimulation was similar in both groups. Finally, adenylate cyclase activity in the presence of forskolin and manganese chloride was similar in both groups. From the above, we conclude that intermittent systemic infusions of ritodrine to pregnant rabbits did not result in down-regulation of myocardial adenylate cyclase activity. (AM J OBSTET GYNECOL 1987;156:749-54.)

Key words: Ritodrine, adenylate cyclase activity, adult rabbit myocardium, desensitization

The stimulating actions of catecholamines on cardiac tissue are primarily, but not exclusively, mediated via β-adrenergic receptors. These in turn regulate contractile and chronotropic responses to stimuli through adenylate cyclase. This enzyme catalyzes the synthesis of 3′,5′-cyclic adenosine monophosphate (3′,5′-cAMP), which acts as a second messenger.[1]

The β-adrenergic receptor–adenylate cyclase complex is thought to consist of at least three protein components: β-adrenergic receptor, stimulatory guanosine triphosphate–binding protein (G_s protein), and adenylate cyclase catalytic activity.[2] When β-adrenergic receptor agonists bind to the β-adrenergic receptor, they promote the formation of high-affinity complex ("ternary" complex of the receptor) with the G-binding protein. In the presence of guanosine triphosphate, the agonists and the β-adrenergic receptor dissociate from the activated G-binding protein, which in turn is thought to interact with a catalytic moiety of the enzyme that leads to adenylate cyclase activation and intracellular 3′,5′-cAMP formation.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Bowman Gray School of Medicine of Wake Forest University.
This work was supported in part by a grant from the American Heart Association, North Carolina Chapter.
This work was presented at the Sixth Annual Meeting of The Society of Perinatal Obstetricians, San Antonio, Texas, January 30–February 1, 1986.
Received for publication April 22, 1986; revised August 4, 1986; accepted October 17, 1986.
Reprint requests: Christos G. Hatjis, M.D., Department of Obstetrics and Gynecology, Bowman Gray School of Medicine, 300 South Hawthorne Road, Winston-Salem, NC 27103.

β-Adrenergic receptor–adenylate cyclase activities are modulated through two major classes of regulatory mechanisms, homologous or heterologous.[3] In the former instance, the regulator is a hormone that normally binds to the β-adrenergic receptor (β-adrenergic receptor agonist or antagonist). Generally, exposure to an agonist may induce a down-regulation (tachyphylaxis) of β-adrenergic receptor–adenylate cyclase function while an antagonist induces an up-regulation. In the latter case, hormones that normally do not bind to β-adrenergic receptors appear to regulate their function indirectly (thyroid hormones, sex steroid hormones, glucocorticoids). Up- or down-regulation may then be induced. Although the exact molecular stoichiometry of these alterations has not been worked out in all systems, there are at least two ways through which the β-adrenergic receptor–adenylate cyclase complex can be modulated: First, the concentration of β-adrenergic receptors in plasma membranes may be dynamically altered; second, the interaction between the β-adrenergic receptor and the G-binding protein might be changed under certain situations, which would in turn be reflected by changes in adenylate cyclase activity.[3, 4]

Ritodrine, a selective β_2-adrenergic receptor agonist, has been widely used for the treatment of premature labor.[5] It has been shown that this drug may have a wide spectrum of cardiovascular side effects ranging from maternal and fetal tachycardia to electrocardiographic changes and congestive heart failure. Some of these cardiovascular effects are mediated through myocardial β-adrenergic receptor function and adenylate

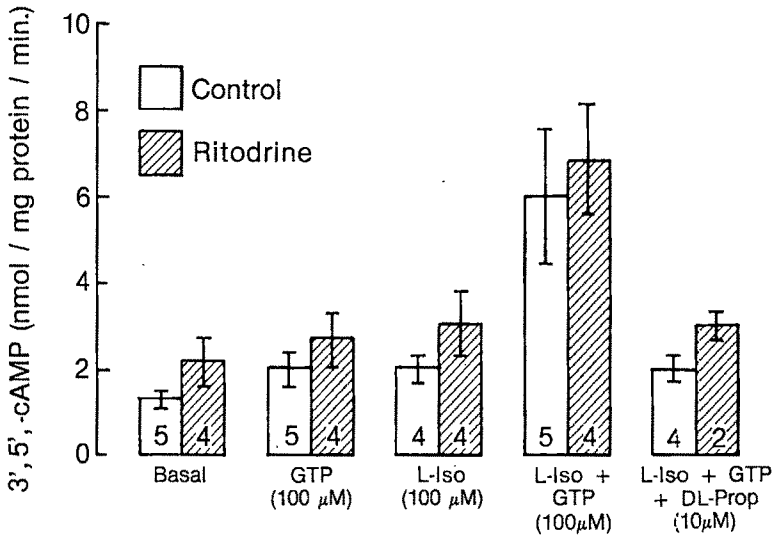


Fig. 1. Basal and stimulated β-adrenergic receptor–dependent myocardial adenylate cyclase activity. *Numbers* in *bars* represent number of individual animals used for that determination. *GTP* = Guanosine triphosphate; *L-Iso* = L-isoproterenol; *DL-Prop* = DL-propranolol.

cyclase activity. Thus it is conceivable that prolonged exposure to ritodrine might result in down-regulation of myocardial β-adrenergic receptor function and adenylate cyclase activity. In this series of experiments we have tried to answer the question whether intermittent administration of ritodrine to pregnant rabbits results in a decrease in myocardial adenylate cyclase activity.

Material and methods

Animals. New Zealand White rabbits with time-dated pregnancies were obtained from a local supplier and housed in our vivarium. At 22 days' gestation (term 31 days) the animals were anesthetized with intramuscular injections of ketamine (30 mg/kg) and xylazine (6 mg/kg). The right jugular vein was then exposed and catheterized with polyvinyl chloride catheters. The catheters were kept patent with infusion of dilute heparin solution in normal saline. The day after operation the animals were randomized into two groups. Group 1, the ritodrine-treated group, received intravenous ritodrine at 7:00 to 8:00 AM and 3:00 to 4:00 PM daily for 3 days. Group 2, the control group, received an equal volume of drug vehicle only (10^{-3}N hydrochloric acid, 0.17 mol/L ethylenediaminetetraacetic acid) according to the same time schedule. At 26 days' gestation, the animals were anesthetized with intravenous pentobarbital sodium (40 mg/kg), following which the doe was put to death. The heart of the animal was then removed in its entirety and separated into ventricles and atria. The position of the jugular catheter was confirmed in the vicinity of the right atrium in all cases. The tissue was then immersed in liquid nitrogen and stored at −70° C until it was used.

The dose of ritodrine received was 5.6 mg/kg of body weight, twice a day. This approximates an average intravenous daily dose of ritodrine for humans (200 μg/min) and represents 1/12 of the 50% lethal dose for rabbits. None of the animals used in these experiments aborted before 27 days' gestation. They were confirmed to be pregnant at the time they were put to death.

Material. Drug and reagents were obtained from commercial sources and were of reagent grade. Ritodrine hydrochloride was provided by Astra Pharmaceuticals. α-^{32}P–labeled adenosine triphosphate (ATP) (10 to 30 Ci/mmol) and 3',8'-^{3}H–labeled 3',5'-cAMP (15 Ci/mmol) were obtained from ICN.

Preparation of heart homogenates.[6] On the day of the assay, the heart ventricle was placed in five to 10 volumes per gram of wet weight of ice-cold 0.9% sodium chloride (w/v) and 20 mmol/L Tris hydrochloride, pH 7.5 (buffer A). It was then minced with scissors and homogenized with a Brinkman Polytron (setting 6-7, 10 seconds). A crude membrane preparation was obtained after centrifugation at 20,000 × g for 10 minutes (4° C). The pellets were then resuspended in 2 ml of ice-cold buffer A and preincubated for 15 minutes at 30° C in the presence of 0.5 mmol/L dithiothreitol, 0.17 mmol/L ethyleneglycol-*bis* (β-aminoethyl ether)-N,N'tetraacetic acid (EGTA), 1 mmol/L hydrochloric acid, 0.2% bovine serum albumin, 5 mmol/L magnesium chloride, pH 7.45. The suspension was then recentrifuged as previously outlined. The resulting pellet was resuspended in ice-cold buffer A (100 to 150 volumes per gram of wet weight) and used in the assay.

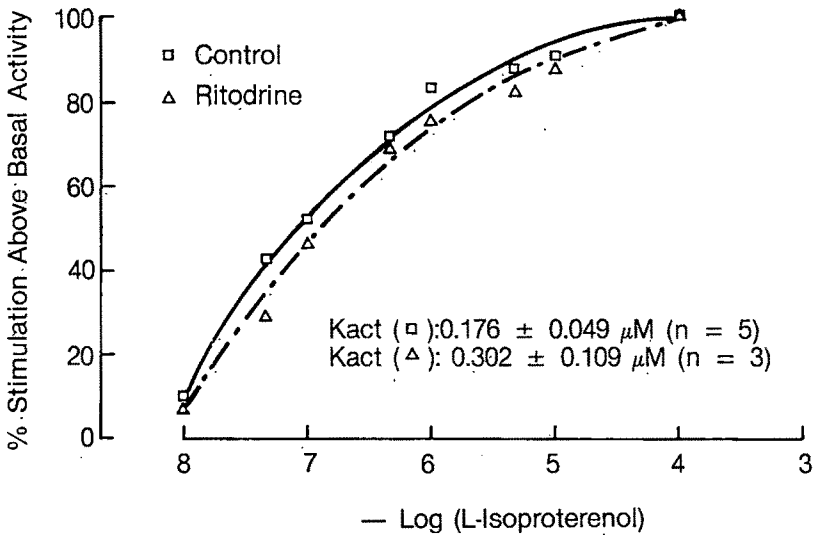


Fig. 2. Dose-response curves for L-isoproterenol–stimulated myocardial adenylate cyclase activity in pregnant adult rabbits. Each point represents the mean of three to five individual determinations.

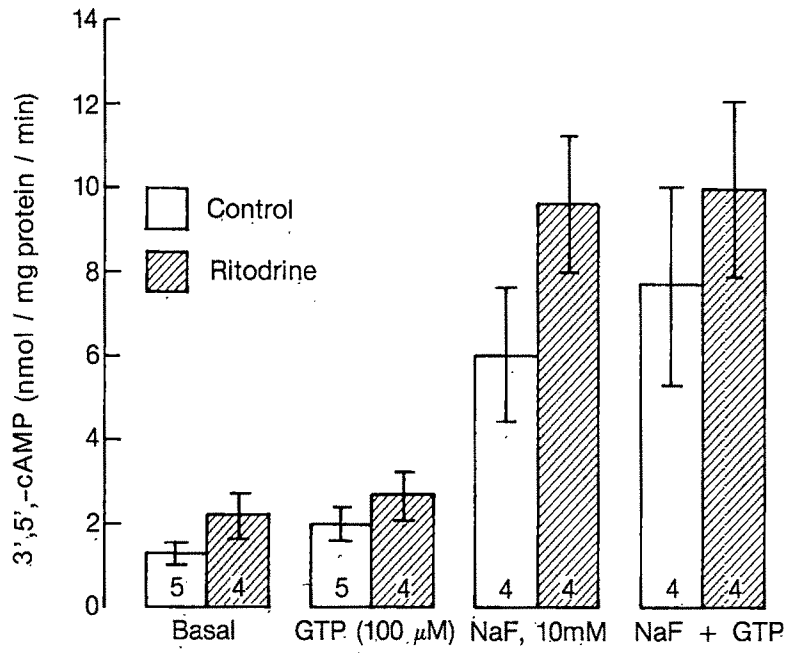


Fig. 3. Basal and stimulated sodium fluoride *(NaF)*–dependent myocardial adenylate cyclase activity. *Numbers* in *bars* represent number of individual animals used for that determination. *GTP* = Guanosine triphosphate.

Adenylate cyclase assay. Adenylate cyclase activity was determined by measuring the conversion of α-^{32}P–labeled ATP to ^{32}P-labeled 3′,5′-cAMP and isolating the product by a modification[6] of a method developed by Salomon.[7] The reaction was carried out in duplicate or triplicate at 30° C for 10 minutes in a final volume of 0.2 ml containing 0.1 ml of membrane fraction (0.23 to 1.3 mg of protein per milliliter) and 50 mmol/L Tris hydrochloric acid, pH 7.5, 0.45% sodium chloride, 0.5 mmol/L adenosine triphosphate, 5 mmol/L magnesium chloride, 0.17 mmol/L EGTA, 0.05 mmol/L 3′,5′-cAMP, 0.5 mmol/L dithiothreitol, 0.75 mmol/L 3-isobutylmethylxanthine, 0.1 mg/ml creatine kinase, 10 mmol/L creatine phosphate, and 1 to 2 × 10^6 cpm of α-^{32}P–labeled ATP. Appropriate drugs were added as required. The reaction was initiated by adding the membrane fraction to the incubation tubes. It was linear with respect to time (20 minutes) and protein concentration. The reaction was terminated by the addition of 0.25 ml of a solution

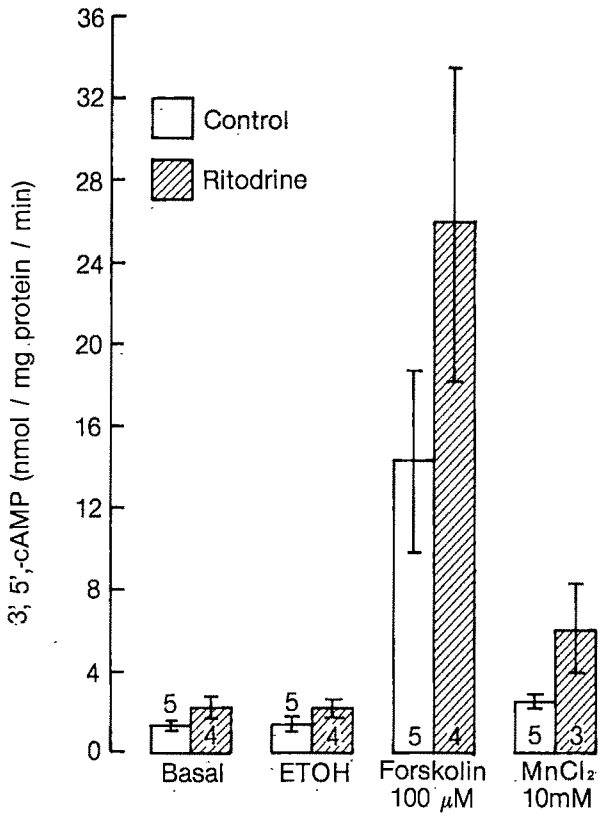


Fig. 4. Basal and stimulated forskolin- and manganese chloride *($MnCl_2$)*–dependent myocardial adenylate cyclase activity in pregnant adult rabbits. Ethyl alcohol *(ETOH)* (6%) was the diluent for forskolin; thus enzymatic activity was determined in its presence (not different from basal activity). *Numbers* in *bars* represent number of individual animals used for that determination.

containing 50 mmol/L Tris hydrochloric acid (pH = 7.5), 5 mmol/L ATP, 1 mmol/L 3′,5′-cAMP, and 5% sodium dodecyl sulfate. ^{3}H-labeled 3′,5′-cAMP (10,000 to 15,000 cpm per tube) was added to all tubes to serve as an internal standard and monitor the efficiency of recovery. The tubes were then placed in boiling water for 10 minutes and allowed to cool down to room temperature. ^{32}P-labeled 3′,5′-cAMP was isolated with sequential Dowex (AG 50W-X4, 200 to 400 mesh, Bio-Rad) and neutral alumina columns and counted in a scintillation counter (Packard). Recovery of 3′,5′-cAMP was determined by the percent recovery of ^{3}H-labeled cAMP external standard in each sample.

Results have been corrected for percent recovery (routinely >60%). Enzymatic activity was expressed as nanomoles of 3′,5′-cAMP produced per milligram of protein per minute (per heart). The K_{act} value of the enzyme for L-isoproterenol (concentration of L-isoproterenol required to half maximally stimulate enzymatic activity) was determined in some experiments by producing dose-response curves. K_{act} was calculated by logit-log transformation of the data from each individual dose-response curve.

Protein assay. Protein concentrations were determined according to the method of Bradford[8] with bovine serum albumin used as a standard.

Statistical analysis. Results presented are expressed as mean ± SEM. We used Student *t* tests to examine the null hypothesis that ritodrine treatment resulted in a decrease in basal and stimulated adenylate cyclase activity. A p value of <0.05 was considered significant.

Results

Adenylate cyclase activity in myocardial membranes was determined under several different experimental conditions. Basal enzyme activity in the presence or absence of guanosine triphosphate did not differ between the two groups (Fig. 1). However, the presence of guanosine triphosphate did increase basal adenylate cyclase activity to a modest degree. β-Adrenergic receptor–mediated adenylate cyclase activity was determined in the presence of maximal L-isoproterenol concentrations (Fig. 1) and by obtaining dose-response curves with variations in the amount of L-isoproterenol present (Fig. 2). L-Isoproterenol–dependent stimulation required the presence of guanosine triphosphate. The degree of stimulation by L-isoproterenol did not differ in the two treatment groups. Moreover, the K_{act} of the enzyme by L-isoproterenol was similar in the two groups of animals tested (Fig. 2). Finally stimulation by L-isoproterenol was specifically inhibited by DL-propranolol, a potent β-adrenergic receptor antagonist (Fig. 1). This series of experiments indicates that adenylate cyclase activity, which is mediated by β-adrenergic receptor stimulation, is not influenced appreciably by previous in vivo exposure to ritodrine.

We next indirectly examined the activity of the G_s-binding protein[2] as indicated by sodium fluoride stimulation (Fig. 3). Again, no significant differences were noted between the control and the ritodrine-treated animals. Sodium fluoride was not dependent on the presence of guanosine triphosphate.

Finally, we indirectly examined the catalytic component of the enzyme by measuring its stimulation in the presence of forskolin and manganese chloride (Fig. 4). We used these compounds since in previous experiments they have been shown to stimulate the catalytic component of the enzyme directly.[9-13] However, in some systems there is evidence to suggest that forskolin might accomplish this stimulation via a process dependent on G_s–binding protein. Forskolin was the most potent stimulator of enzymatic activity (Fig. 4). Addition of guanosine triphosphate to forskolin did not change the degree of adenylate cyclase stimulation (unpublished observations). Although manganese chloride stimulated enzymatic activity appreciably,

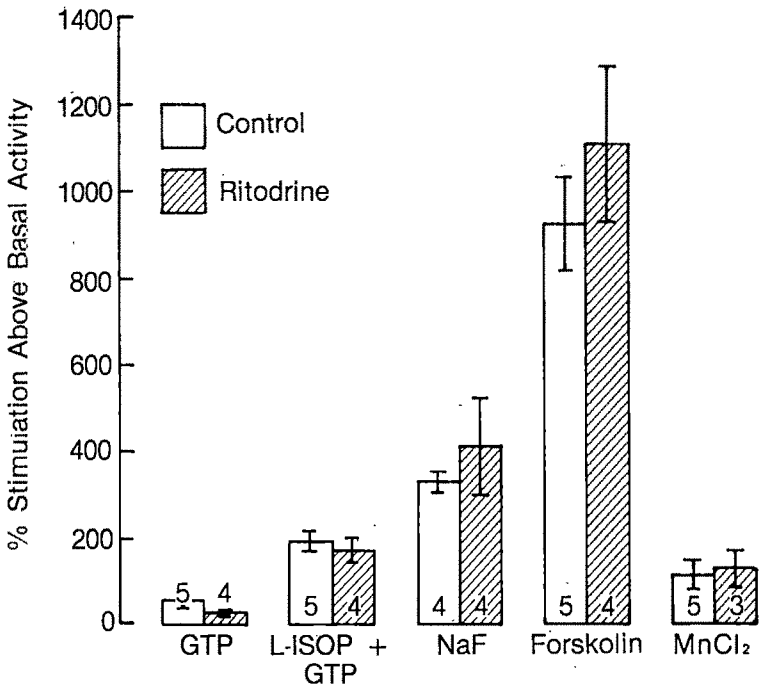


Fig. 5. Adenylate cyclase stimulation in myocardium of pregnant adult rabbits. Data from Figs. 1 to 4 have been normalized with regard to basal activity. No significant differences were noted. *Numbers* in *bars* represent number of individual animals used for that determination. *GTP* = Guanosine triphosphate; *L-ISOP* = L-isoproterenol; *NaF* = sodium fluoride; *$MnCl_2$* = manganese chloride.

it was significantly less potent than forskolin (Fig. 4).

Fig. 5 shows the percent activation over basal enzymatic activity by guanosine triphosphate, L-isoproterenol, sodium fluoride, forskolin, and manganese chloride. Forskolin was the most potent stimulant. However, the degree of stimulation by each of these agents did not differ between the control and ritodrine-treated animals.

In preliminary experiments we documented that the ritodrine hydrochloride we used to inject the animals did have in vitro activity in terms of both displacing ^{125}I-labeled iodohydroxybenzylpindolol from β-adrenergic receptors in myometrial membranes[14] and stimulating adenylate cyclase activity in myocardial membranes in the presence of guanosine triphosphate (unpublished observations). These experiments indicate that the ritodrine the animals received had not been inactivated.

Comment

Ritodrine is a potent selective β_2-adrenergic receptor agonist. However, it is similar to the other existing β-sympathomimetic drugs in that it acts to some degree on both β_1- and β_2-adrenergic receptors. The predominant subtype in ventricular myocardium is the β_1-adrenergic receptor.[4, 15] Thus it is not unusual to observe significant cardiovascular side effects as a result of ritodrine treatment. It has been shown[5] that ritodrine infusion results in significant maternal tachycardia and in a decrease in stroke volume and possibly in stroke work index. Finally, in some instances ritodrine has been associated with chest pain, congestive heart failure, and evidence of myocardial ischemia.

We hypothesized that in vivo exposure to ritodrine might result in down-regulation of myocardial β-adrenergic receptor function as manifested by adenylate cyclase activity in myocardial membranes from the adult pregnant rabbit.

To answer the question, we performed in vitro experiments in which we measured adenylate cyclase activity in ventricular heart membranes from pregnant rabbits. We found that daily intermittent treatment of rabbits with ritodrine did not result in any appreciable changes in myocardial adenylate cyclase activity. We did not see any changes in β-adrenergic receptor–mediated enzymatic activity nor did we observe an indirect effect on the function of G_s-binding protein or on the catalytic component of the complex. Thus under these experimental conditions ritodrine does not result in down-regulation of adenylate cyclase activity.

These results are somewhat in conflict with previously reported observations after isoproterenol treatment of animals where down-regulation of myocardial receptors had indeed been observed.[16] In that study, although L-isoproterenol was administered intermittently, the duration of treatment was for 10 days. How-

ever, desensitization has been reported even after a short-term infusion of L-isoproterenol. These results suggest that ritodrine may behave somewhat differently than isoproterenol and that it may affect only tissues that contain primarily β_2-adrenergic receptors. Finally, whether continuous, rather than intermittent, long-term infusion of ritodrine might result in desensitization of myocardial β-adrenergic receptors is not known.

Ritodrine has profound metabolic effects that might influence myocardial function regardless of β-adrenergic receptor and adenylate cyclase activity. Specifically, it has been associated with hypokalemia and hypocalcemia,[5] derangements that might affect myocardial cell repolarization and contractility. Thus it is possible that if ritodrine administration does not affect adenylate cyclase function in myocardial membranes, it might compromise it through secondary metabolic effects. Further work in this area to delineate the action of this drug with regard to myocardial performance is warranted.

REFERENCES

1. Stull JT, Mayer SE. Biochemical mechanisms of adrenergic and cholinergic regulation of myocardial contractility. In: Berne RM, ed. Handbook of physiology. Bethesda, Maryland: American Physiological Society, 1979, vol 1, sect 2:581.
2. Birnbaumer L, Codina J, Mattera R, et al. Regulation of hormone receptors and adenylyl cyclases by guanine nucleotide binding–proteins. Recent Prog Horm Res 1986; 41:41.
3. Stiles GL, Caron MG, Lefkowitz RJ. β-Adrenergic receptors: biochemical mechanisms of physiological regulation. Physiol Rev 1984;64:661.
4. Stiles GL, Lefkowitz RJ. Cardiac adrenergic receptors. Ann Rev Med 1984;35:149.
5. Lipshitz J. Beta-adrenergic agonists. Semin Perinatol 1981;5:252.
6. Hatjis CG. Adenylate cyclase activity in fetal rabbit myocardium. Pediatr Res 1985;19:75.
7. Salomon Y, Londos C, Rodbell M. A highly sensitive adenylate cyclase assay. Anal Biochem 1974;58:541.
8. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72:248.
9. Hatjis CG. Forskolin-stimulated adenylate cyclase activity in fetal and adult rabbit myocardial membranes. Am J Obstet Gynecol 1986;155:1326.
10. Daly JW. Forskolin, adenylate cyclase, and cell physiology: an overview. In: Greengard P, Robison GA, Paoletti R, Nicosia S, eds. Advances in cyclic nucleotide and protein phosphorylation research. New York: Raven Press, 1984, vol 17:81.
11. Braun T, Frank H, Dods R, Sepsenwol S. Mn^{2+} sensitive, soluble adenylate cyclase in rat testis: differentiation from other testicular nucleotide cyclases. Biochim Biophys Acta 1977;481:227.
12. Hildebrandt JD, Sekura RD, Codina J, Iyengar R, Manclark CR, Birnbaumer L. Stimulation and inhibition of adenylyl cyclases mediated by distinct regulatory proteins. Nature 1983;302:706.
13. Stengel D, Guenet L, Desmier M, Insel P, Hanoune J. Forskolin requires more than the catalytic unit to activate adenylate cyclase. Mol Cell Endocrinol 1982;28:681.
14. Hatjis CG. β-Adrenergic–receptor and adenylate cyclase properties in pregnant and nonpregnant guinea pig myometrium. Am J Obstet Gynecol 1985;151:943.
15. Hatjis CG, McLaughlin MK. Identification and ontogenesis of β-adrenergic receptors in fetal and neonatal rabbit myocardium. J Dev Physiol 1982;4:327.
16. Tse J, Powell JR, Baste CA, Priest RE, Kuo JF. Isoproterenol-induced cardiac hypertrophy: modifications in characteristics of β-adrenergic receptor, adenylate cyclase, and ventricular contraction. Endocrinology 1979; 105:246.

A complete list of references is available on request from the authors.

Infusion of gonadotropin-releasing hormone agonist during pregnancy: Maternal and fetal responses in primates

V. M. Sopelak, Ph.D., and G. D. Hodgen, Ph.D.
Jackson, Mississippi, and Norfolk, Virginia

To determine whether a gonadotropin-releasing hormone agonist could cross the placenta to the fetus, each pregnant rhesus monkey (110 to 155 days' gestation, n = 10) and her in utero fetus had indwelling cannulas placed in the femoral veins. Gonadotropin-releasing hormone agonist (1000 μg intravenously) was injected into the mother (n = 8) or fetus (n = 2); serial blood samples were collected from mother and fetus for luteinizing hormone and follicle-stimulating hormone determination. None of the mothers responded to the gonadotropin-releasing hormone agonist bolus. In contrast, some of the fetal monkeys receiving gonadotropin-releasing hormone agonist via placental transfer and all fetal monkeys injected directly with the agonist responded as indicated by increased luteinizing hormone (2.8- to 12.3-fold) and follicle-stimulating hormone (1.5- to 8.9-fold) concentrations. In evaluating maternal and fetal effects, we found that continuous maternal infusion with either gonadotropin-releasing hormone agonist (n = 14) or saline solution (n = 11) throughout pregnancy did not alter maternal hormonal profiles. Histologically, gonads from their infants, removed within 3 days post partum, were normal. Although neonatal ovarian weights were unaffected by the in utero treatment, the testes weighed less ($p < 0.05$) in male infants born of mothers treated with gonadotropin-releasing hormone agonist compared to controls. Thus gonadotropin-releasing hormone agonist can cross from maternal to fetal circulation, and the fetus can respond (at least during the third trimester). (Am J Obstet Gynecol 1987;156:755-60.)

Key words: Gonadotropin-releasing hormone agonist, pregnancy, primates, gonadal development, gonadotropic hormones

Pulsatile administration of native gonadotropin-releasing hormone (GnRH) or other regimens for its agonistic analogs are being used increasingly in endocrine therapeutics.[1,2] Chronic treatment of experimental animals or humans with native GnRH or its synthetic agonistic analogs leads to decreased pituitary secretory responses and gonadal suppression in both male and female subjects.[1,3-5] Thus long-acting GnRH agonists are being evaluated for multiple medical indications, including prostatic cancer, endometriosis, contraception in women, uterine myeloma, breast cancer, and ovulation induction.

As with any medical agent, there is always a risk of pregnancy occurring during therapy in women of reproductive age. It is noteworthy that administration of GnRH or its agonistic analogs may initially evoke increases in luteinizing hormone and follicle-stimulating hormone secretion in adults, followed by down-regulation of the gonadotropes leading to a reduction of the total concentrations of follicle-stimulating hormone and luteinizing hormone.[4] If GnRH agonists pass easily from maternal circulation through the placenta to the fetus, there is concern about potential effects on fetal development, especially the gonadal and sex organ formative processes.

Studies using primates have shown that ablation of the fetal hypophysis leads to disorganized oogenesis and degeneration of an increased percentage of germ cells in the female neonate and a reduction in infant testes weights, percentage of Leydig cells, and spermatogonia per testis in infant primate males.[6,7] In anencephalic human fetuses surviving until term, hypoplasia of the ovaries and failure of normal progression of follicular maturation occurs.[8] Since these fetuses have less gonadotropin secretion resulting from a deficiency of pituitary function, it is thought that hypoplastic ovaries in neonates can result from lack of adequate gonadotropic stimulation during intrauterine life. Thus inadequate gonadotropic stimulation resulting from disease or surgery or brought about by drugs transferred across the placenta may disrupt normal fetal gonadal development.

From the Department of Obstetrics and Gynecology, University of Mississippi Medical Center, and the Jones Institute for Reproductive Medicine, Department of Obstetrics and Gynecology, Eastern Virginia Medical School.
Supported in part by Ford Foundation Grant 810-0293 and by the Vicksburg Hospital Medical Foundation.
Presented at the Sixty-seventh Annual Meeting of the Endocrine Society, Baltimore, Maryland, June 19-21, 1985.
Received for publication June 16, 1986; revised September 29, 1986; accepted October 27, 1986.
Reprint requests: Dr. V. M. Sopelak, Department of Obstetrics and Gynecology, University of Mississippi Medical Center, 2500 North State St., Jackson, MS 39216.

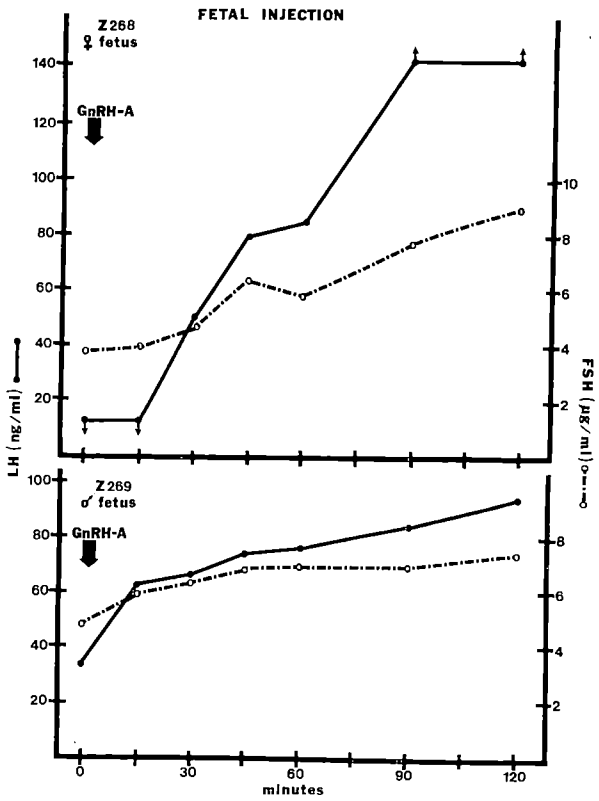


Fig. 1. Hormonal profiles of luteinizing and follicle-stimulating hormone secretion in a female and male fetus following an intravenous bolus injection of 1000 μg of a GnRH agonist (D-tryptophan[6], proline[9], GnRH). Note that the GnRH agonist elicits an increase in both gonadotropins within 30 minutes after injection.

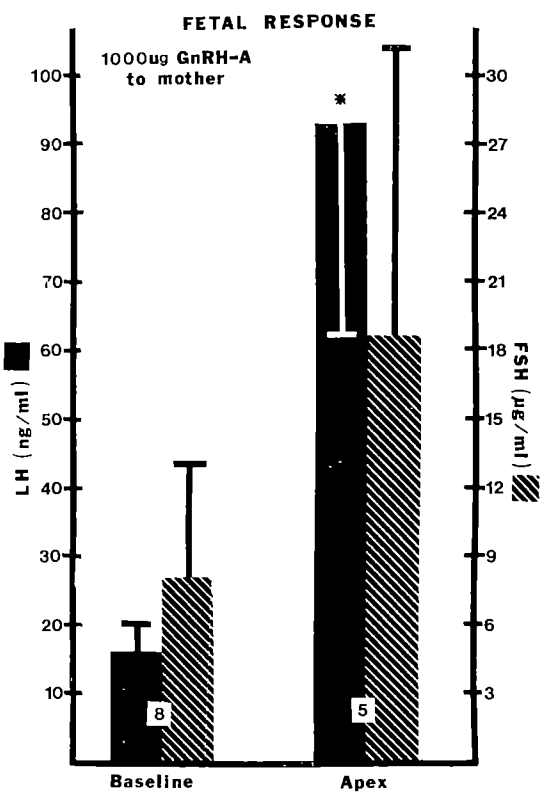


Fig. 2. Fetal gonadotropin response to maternal intravenous injection of 1000 μg of GnRH agonist. *Baseline* (mean ± SEM) levels of luteinizing and follicle-stimulating hormones are depicted for all eight fetal monkeys, while the highest concentrations of gonadotropins after GnRH agonist injection *(apex)* represent five fetal animals showing a response. These data indicate that GnRH agonist can traverse from maternal to fetal circulation via placental transfer.

In the present study we examined (1) whether a GnRH agonist, administered to the primate mother, would affect maternal hormonal levels; (2) whether this agonist would cross the placenta and stimulate fetal pituitary secretion of follicle-stimulating hormone and luteinizing hormone; (3) whether fetal gonadal development is affected morphologically; (4) whether postnatal pituitary response to castration is affected.

Material and methods

Animals. Female rhesus monkeys *(Macaca mulatta)* were caged individually in a controlled environment (temperature, 75° to 80° C; humidity, 60% minimum; lights on from 5 AM to 5 PM). The monkeys were given water ad libitum, fed a commercial monkey chow twice daily, and maintained with use of standard husbandry practices. From days 11 to 14 of the menstrual cycle, the female monkeys cohabitated with male ones of proven fertility. Conception was confirmed by a urinary nonhuman primate pregnancy test for chorionic gonadotropin[9] (provided by the Center for Population Research, National Institute of Child Health and Human Development, Bethesda, Maryland) performed on days 19 and 21 after mating and by palpation of the reproductive tract on day 35.

Study I. Placental transfer of GnRH agonist. To determine whether a potent GnRH agonist (D-tryptophan[6], proline[9] Net–luteinizing-releasing factor–ethylamide; provided by the Contraceptive Development Branch, National Institute of Child Health and Human Development) would cross the placenta and whether the fetal pituitary could respond to the GnRH agonist, each pregnant monkey (110 to 155 days' gestation, n = 10) and her in utero fetus was cannulated via the femoral vein after providing ketamine hydrochloride anesthesia (75 mg intramuscularly, Parke-Davis, Inc., Morris Plains, New Jersey). GnRH agonist (1000 μg in saline solution intravenously) was injected into the mother (n = 8) or fetus (n = 2). Blood (3.0 ml) was simultaneously collected from mother and fetus at 0, 15, 30, 45, 60, 90, and 120 minutes. Erythrocytes in saline solution were returned to each fetal monkey after the hourly samples were collected. Sera were harvested and stored frozen (−20° C) until radioimmunoassays

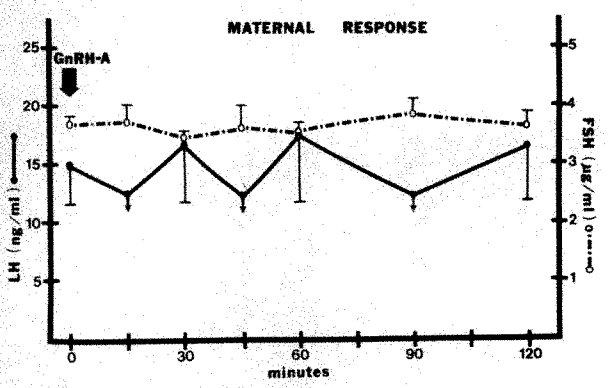


Fig. 3. Profiles of maternal luteinizing and follicle-stimulating hormone secretion (mean ± SEM) following a 1000 μg intravenous bolus of GnRH agonist. The steady state of gonadotropin concentrations are indicative of a pituitary that is unresponsive to GnRH agonist stimulation during pregnancy.

were performed for measurement of estradiol, progesterone, luteinizing hormone, and follicle-stimulating hormone as previously described.[10, 11] The R_{13} antiserum used for radioimmunoassay of monkey luteinizing hormone cross reacts with human luteinizing hormone, human chorionic gonadotropin, and comparable gonadotropins.

Study II. Continuous GnRH agonist infusion during pregnancy. GnRH agonist (6.6 μg/hr) was continuously administered to pregnant rhesus monkeys via a subcutaneous osmotic pump[12] (Model 2ML4; Alza Corp., Palo Alto, California) starting either during the first trimester (day 35 to term, n = 5) or third trimester (day 100 to term, n = 9). The osmotic pumps contained a 30-day supply of the agonist and were replaced at 28-day intervals. Control primates (n = 11) received saline solution (2.5 μl/hr). Maternal blood samples were collected every third day by venipuncture under ketamine hydrochloride anesthesia. Samples were frozen until assayed for estradiol, progesterone, luteinizing hormone, and follicle-stimulating hormone concentration. After spontaneous term delivery and within 3 days post partum, each infant monkey was weighed and anesthetized; its gonads were removed, weighed, fixed, serially sectioned, and stained with hematoxylin and eosin as previously described.[6, 7] A blood sample was obtained from each infant just before castration followed by weekly samples until 3 months of age. Sera were analyzed for concentrations of luteinizing hormone and follicle-stimulating hormone as previously described.[11] All samples from an individual monkey were analyzed in the same assay.

Data were compared with use of a one-way analysis of variance to determine whether ovarian weights, testis weights, and hormonal levels were different between infants born to saline solution–treated control monkeys compared to mothers treated with GnRH agonist. Since

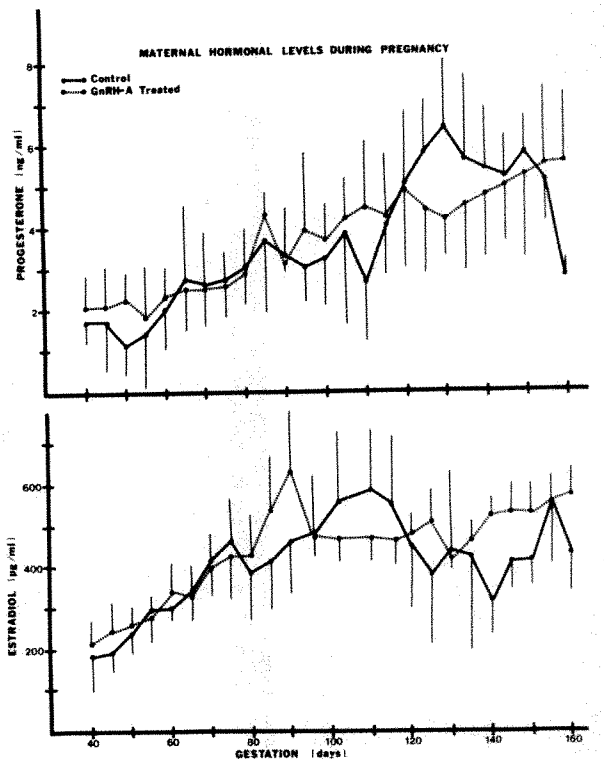


Fig. 4. Maternal hormonal profiles (mean ± SEM) of estradiol and progesterone secretion during pregnancy are unaltered from those of control primates, even when a GnRH agonist (6.6 μg/hr) is continuously administered during pregnancy via a subcutaneous osmotic pump.

such data from monkeys beginning treatment during the first or third trimester of pregnancy were statistically similar, the neonatal hormonal and gonadal data were combined and analyzed with respect to GnRH agonist treatment versus control.

Results

A bolus of GnRH agonist, when injected directly into the fetoplacental circulation in utero, elicited a prompt rise in fetal primate gonadotropin concentrations (Fig. 1). Similarly, when the agonist was injected into the maternal circulation, five of eight fetuses responded with a significant elevation of serum luteinizing hormone ($p < 0.01$) and a less dramatic rise in follicle-stimulating hormone concentration (Fig. 2). The remaining three fetal monkeys (>155 days of gestation) maintained gonadotropin values at the baseline. Thus placental transfer of the agonist did occur as judged by a rise in fetal gonadotropin levels in fetoplacental circulation. In contrast, none of the primate mothers showed any significant fluctuation in luteinizing hormone or follicle-stimulating hormone secretion following administration of this bolus of GnRH agonist (Fig. 3).

Maternal hormonal profiles of estradiol, progester-

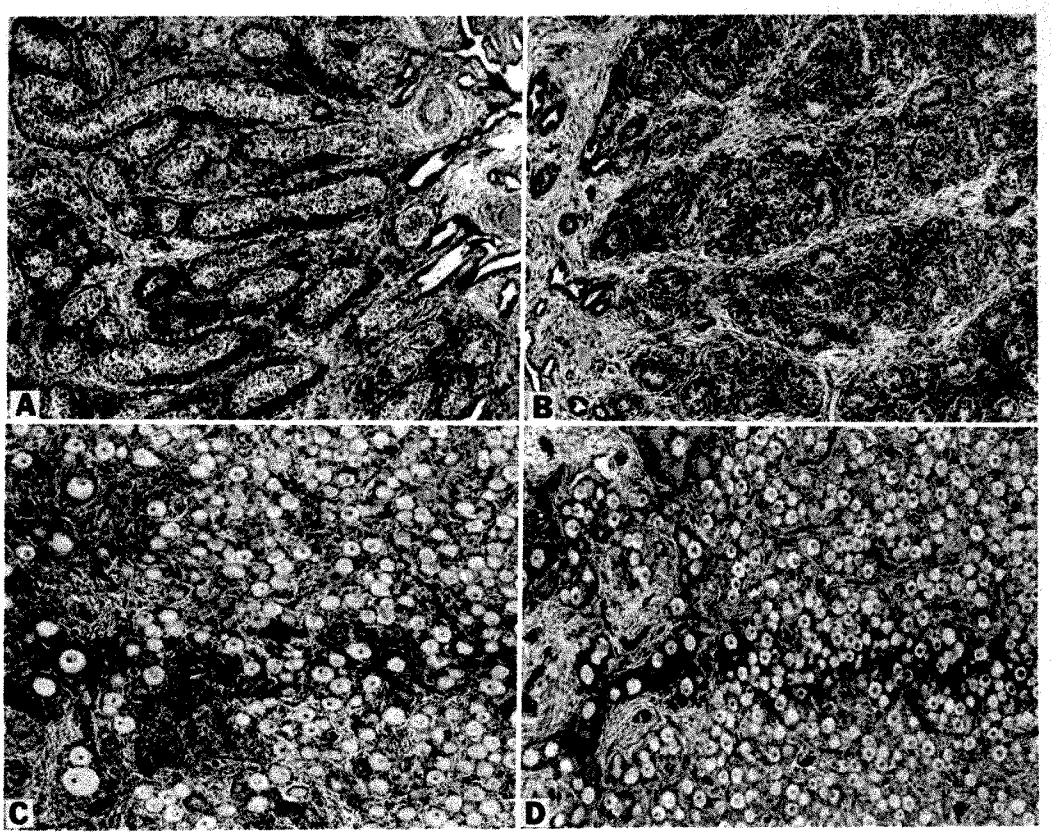


Fig. 5. Representative histologic sections of testis from a control infant *(A)* and a neonate whose mother was continuously treated during pregnancy with a GnRH agonist *(B)* are structurally similar. Sections of ovary from a control infant *(C)* and female neonate whose mother had received 6.6 μg/hr of GnRH agonist during pregnancy *(D)* are indistinguishable from each other.

one, luteinizing hormone, and follicle-stimulating hormone secretion were similar, irrespective of whether saline solution or GnRH agonist had been continuously administered throughout pregnancy (Fig. 4). The mothers treated with GnRH agonist maintained viable pregnancies (162.7 ± 1.9 days' gestation) and spontaneously delivered mature infants near normal term.[13]

At the time of delivery the genitalia of infants born from mothers treated continuously from either the first or third trimester with the GnRH agonist were normal in appearance. Histologically the gonads from treated and control neonates were indistinguishable (Fig. 5). However, testis weights were lighter ($p < 0.05$) in both groups of male infants treated with GnRH agonist, irrespective of whether treatment had started during the first or third trimester of pregnancy compared to controls (Table I). The differences in testis weight were not associated with differences in infant body weight (control vs. GnRH-treated male infants: 375 ± 30 gm vs. 415 ± 17 gm, respectively) or to significant differences in circulating gonadotropins (Table I), as measured within 3 days after birth. In contrast, neonatal ovarian weights were unaffected by the in utero infusion of the GnRH agonist. Luteinizing hormone and follicle-stimulating hormone concentrations were similar ($p > 0.05$) at the time of birth, irrespective of whether each female infants' mother received saline solution or GnRH agonist during pregnancy.

After castration, all treated and control infant monkeys responded to castration, manifesting steadily rising gonadotropin levels. By 5 weeks of age, gonadotropin values had risen from <15 ng/ml for luteinizing hormone and 2.3 ± 0.2 μg/ml for follicle-stimulating hormone in both male and female control infants to levels of 71.1 ± 24.8 ng/ml of luteinizing hormone and 24.1 ± 3.4 μg/ml of follicle-stimulating hormone. Similarly, castrate male and female infants born of mothers treated with GnRH agonist showed a progressive increase in luteinizing hormone (17.3 ± 2.1 to 115.7 ± 17.6 ng/ml) and follicle-stimulating hormone (4.3 ± 1.2 to >27 μg/ml) by 5 weeks of age ($p < 0.05$). Thus response of the pituitary to postnatal castration was not modified by in utero exposure to continuous GnRH agonist infusion.

Table I. Gonadal weights and hormonal values in newborn monkeys

	n	*Weight of gonad (mg)*	*Follicle-stimulating hormone (μg/ml)*	*Luteinizing hormone (ng/ml)*
Female				
Control	7	36.7 ± 3.8	2.1 ± 0.2	<15
GnRH agonist–treated	4	32.0 ± 2.8	6.1 ± 4.0	20.7 ± 7.5
Male				
Control	4	57.1 ± 5.4	2.8	<15
GnRH agonist–treated	6	42.9 ± 3.5*	3.3 ± 0.5	15.4 ± 0.5

*$p < 0.05$.

Comment

In this study we demonstrate that a GnRH agonist crosses from maternal to fetal circulation, often eliciting an increased pituitary-gonadotropin response from the in utero monkey as measured during the third trimester. That the luteinizing hormone response is of fetal rather than placental origin is plausible, since monkey chorionic gonadotropin is not detectable in serum, urine, or placental extracts beyond day 40 of pregnancy in the rhesus monkey,[14] there was a concomitant rise in fetal follicle-stimulating hormone after GnRH agonist injection, and there was no gonadotropin rise observed in maternal circulation. The findings of a fetal response to the GnRH agonist are consistent with our earlier preliminary data which showed that peptide hormones of 10 or 12 amino acids, such as native GnRH, could readily cross the placenta.[15] We are not surprised that three of eight in utero fetal primates did not respond to the maternal injection of GnRH agonist. Decreased placental blood flow caused by manipulation of the uterus and fetus during sample collection may have been a contributing factor. More important, however, may be the influence of gestational age. It is known that the responsiveness of fetal monkeys to in utero GnRH injections declines from day 100 to 160 of gestation.[16] Notice that all three unresponsive fetal monkeys in our study were of >155 days' gestation, whereas the five responders were less advanced.

Neither a bolus of GnRH agonist administered to the mother nor continuous GnRH agonist infusion throughout pregnancy altered maternal hormonal profiles. Other investigators have reported that both the human and monkey pituitary are unresponsive to acute GnRH stimulation during pregnancy.[15, 17] Elevated levels of steroid hormones (such as estradiol and progesterone) have been implicated in the refractoriness of the hypophysis to GnRH and its agonists during pregnancy. Although there have been reports that GnRH may induce increased secretion of chorionic gonadotropin from the placenta in the human, that mechanism is not well fitted to our observations in the rhesus monkey.

Since the maternal hormonal levels monitored during pregnancy were unaffected by continuous administration of the GnRH agonist, we postulate that any effect on the fetus may be due to changes in fetal physiology rather than to an altered maternal hormonal milieu. The cause of the reduction in testis weights of treated infants in this study is unknown. There were no concomitant reductions in infant body weight or a significant alteration in the architecture of the neonatal testis. Thus the effects of the GnRH agonist were not like those observed after surgical fetal hypophysectomy, in which the percentage of Leydig cells and spermatogonia per testis was reduced.[6, 7] Histologically the gonads of male infants exposed to GnRH agonist throughout gestation did not seem adversely affected.

The ovarian weights of female infants were unaffected by the GnRH agonist regimen. This finding should be contrasted with effects of fetal hypophysectomy during gestation which significantly reduced ovarian weight and germ cell number,[6] not unlike the results reported in male infants.[7] Although in utero exposure to the GnRH agonist was begun in some animals before completion of internal and external gonadal differentiation,[18] the genitalia of male and female infants were both normal. Pituitary response to castration in both male and female infants was in accordance with earlier results;[19] soon gonadotropin concentrations in castrate infant monkeys approached levels seen in castrate adult females.[20] Infants born of control mothers responded to castration in a similar fashion.

In conclusion, we have demonstrated that a long-acting GnRH agonist can easily cross from maternal circulation to the fetoplacental compartment in which the fetal pituitary responded (follicle-stimulating hormone/luteinizing hormone) to the agonist during the third trimester of pregnancy. Meanwhile, hormonal levels in the primate mother were unaffected. Despite a slight suppression of testicular weight at term, this GnRH agonist did not prevent virilization of males or induce ambiguous genitalia among female monkeys although administration in some monkeys was begun before internal and external gonadal differentiation was complete. Although we cannot comment on the potential teratogenic effects of GnRH agonist administration

before day 35 of pregnancy, we conclude that fetal in utero exposure after day 35 has little effect on overt gonadal development. However, longer-term follow-up studies are required to extend the level of safety suggested here, ultimately including demonstration of normal pubertal development and adult fertility.

We wish to thank the former members of the Pregnancy Research Branch, National Institutes of Health, for their helpful discussions and technical assistance. In addition, we are grateful to Dr. G. Bialy for providing the GnRH agonist used in this study, to the National Hormone and Pituitary Program for monkey follicle-stimulating hormone and luteinizing hormone kits, to Dr. W. Wiser, and to the faculty and staff of the Department of Obstetrics and Gynecology, University of Mississippi, for their research support.

REFERENCES

1. Yen SSC. Clinical applications of gonadotropin-releasing hormone and gonadotropin-releasing hormone analogs. Fertil Steril 1983;39:257.
2. Hodgen GD. Releasing hormones as diagnostic and therapeutic agents. Fertil Steril 1983;39:592.
3. Corbin A, Beattie CW, Tracy J, et al. The anti-reproductive pharmacology of LH-RH and agonistic analogues. Int J Fertil 1978;23:81.
4. Meldrum DR, Chang RJ, Lu J, Vale W, Rivier J, Judd HL. "Medical oophorectomy" using a long-acting GnRH agonist: a possible new approach to the treatment of endometriosis. J Clin Endocrinol Metab 1982;54:1081.
5. Warner B, Worgul TJ, Drago J, et al. Effect of very high dose D-leucine^6-gonadotropin-releasing hormone proethylamide on the hypothalamic-pituitary testicular axis in patients with prostatic cancer. J Clin Invest 1983; 71:1842.
6. Gulyas BJ, Hodgen GD, Tullner WW, Ross GT. Effects of fetal or maternal hypophysectomy on endocrine organs and body weight in infant rhesus monkeys (Macaca mulatta): with particular emphasis on oogenesis. Biol Reprod 1977;16:216.
7. Gulyas BJ, Tullner WW, Hodgen GD. Fetal or maternal hypophysectomy in rhesus monkeys (Macaca mulatta): effects on the development of testes and other endocrine organs. Biol Reprod 1977;17:650.
8. Ross GT, Vande Wiele RL. Part I: The ovaries. In: Williams RH, ed. Textbook of endocrinology. 6th ed. Philadelphia: WB Saunders, 1981:355.
9. Hodgen GD, Ross GT. Pregnancy diagnosis by hemagglutination inhibition test for urinary macaque chorionic gonadotropin (mCG). J Clin Endocrinol Metab 1974; 38:927.
10. Hodgen GD, Wilks JW, Vaitukaitis JL, Chen HC, Papkoff H, Ross GT. A new radioimmunoassay for follicle-stimulating hormone in macaques: ovulatory menstrual cycles. Endocrinol 1976;99:137.
11. Sopelak VM, Collins RL, Hodgen GD. Follicular stimulation versus ovulation induction in juvenile primates: importance of gonadotropin-releasing hormone dose. J Clin Endocrinol Metab 1986;62:557.
12. Sopelak VM, Hodgen GD. Techniques for research in fetal and neonatal primates. In: Nathanielsz PW, ed. Animal models in fetal medicine (III). New York: Perinatology Press, 1984:161.
13. Hodgen GD, Dufau ML, Catt KJ, Tullner WW. Estrogens, progesterone and chorionic gonadotropin in pregnant rhesus monkeys. Endocrinol 1972;91:896.
14. Hodgen GD, Niemann WH, Tullner WW. Duration of chorionic gonadotropin production by the placenta of the rhesus monkey. Endocrinol 1975;96:789.
15. Lundblad EG, Williams RF, Chen HC, Hodgen GD. Placental passage of protein hormones in rhesus monkeys [Abstract 31]. In: Scientific Abstracts of the twenty-seventh annual meeting of the Society for Gynecologic Investigation, Denver, Colorado, March 19-22, 1980. Denver, Colorado: Society for Gynecologic Investigation, 1980:20.
16. Yonekura ML, Williams RF, Hodgen GD. Changing pituitary response to gonadotropin releasing hormone in fetal and infant primates [Abstract 208]. In: Scientific Abstracts of the twenty-seventh annual meeting of the Society for Gynecologic Investigation, Denver, Colorado, March 19-22, 1980. Denver, Colorado: Society for Gynecologic Investigation, 1980:125.
17. Jeppsson S, Rannevik G. Studies on the gonadotropin response after administration of LH/FSH-releasing hormone (LRH) during pregnancy and after therapeutic abortion in the second trimester. Am J Obstet Gynecol 1976;125:484.
18. van Wagenen G, Simpson ME. Embryology of the ovary and testis: *Homo sapiens* and *Macaca mulatta*. New Haven, Connecticut: Yale University Press, 1965:19.
19. Williams RF, Hodgen GD. The reproductive cycle in female macaques. Am J Primatol 1982;1(suppl):181.
20. Schenken RS, Asch RH, Anderson WH. Effect of porcine follicular fluid on the postcastration secretion of gonadotropins in rhesus monkeys. J Clin Endocrinol Metab 1984;59:436.

CORRESPONDENCE

Müllerіosis instead of endometriosis

To the Editors:

Sanfilippo et al. (AM J OBSTET GYNECOL 1986;154: 39-43) present a case report of alleged endometriosis associated with müllerian anomaly. It is unclear from the diagram in their Fig. 1 how menstrual blood could escape from the fallopian tube into the abdominal cavity if the patient had a hematosalpinx on the right side. The foci of endometriosis were not sampled before or after their surgical treatment, so we do not know whether or not they really represented endometriosis. It is also unclear how they come to the conclusion that this was stage IV endometriosis, since higher stages of the American Fertility Society Classification require extensive adhesions, which were not shown in Fig. 1. It is unusual that they present two additional cases of müllerian anomaly, also without any objective proof that endometriosis was present. It will serve only to further confuse the literature on this important disease to let these loose clinical observations go unchallenged into the literature.

It is interesting to note that the relationship that the authors tried to develop between müllerian anomalies and supposed reflux menstruation and implantation of endometriosis has a parallel etiology that is possible. This and all previous reports describing this presumed association are equally strong arguments for "müllerіosis." Müllerіosis refers to a developmental defect of differentiation or migration of the müllerian duct system during embryology, with the resultant findings of displaced müllerian tissue in the adult manifested as endometriosis, endocervicosis, accessory fimbria, hydatids of the tube, cervical stenosis, as well as fusion defects of the uterus, cervix, or vagina. Endometriosis just happens to be the most common of these birth defects. Until this etiologic possibility is realized, we will continue to see articles jousting with the windmill of reflux menstruation.

David B. Redwine, M.D.
Mountain View Women's Clinic, P. C.
2381 N. E. Connors Avenue
Bend, Oregon 97701

Reply

To the Editors:

We are sorry that our diagram was unclear. There was significant intraperitoneal bloody effluent from the hematosalpinx. It is this effluent that we propose as being the source of the endometriosis.

The disease is described as having "deep ovarian involvement, partial obliteration of the cul-de-sac, and two-thirds enclosure of the ovary with dense and filmy adhesions." This scores 44 points (severe) on the American Fertility Society classification of endometriosis. Although biopsy evidence was not available in each case, it was available in case 2. There has been an additional case since publication of the original article in which biopsy evidence of endometriosis has also been established.

We appreciate Redwine's alternate explanation for our findings. His explanation, however, fails to address the two major points of our paper: (1) Spontaneous regression of the endometriosis occurred after elimination of the obstruction to menstrual flow in these patients. (2) Since spontaneous regression obviously does not occur in most patients with endometriosis, the entity we described after menstrual obstruction either is different from classic endometriosis or indicates that a permissive factor (such as immune incompetence) must work in concert with the reflux to present phagocytosis of the debris in most cases.

Joseph Sanfilippo, M.D.
Department of Obstetrics and Gynecology
University of Louisville
550 S. Jackson Street
Louisville, Kentucky 40292

Nurse-midwifery care and improved outcomes

To the Editors:

In their article entitled "The perinatal and economic impact of prenatal care in a low socioeconomic population" (AM J OBSTET GYNECOL 1985;154:1), Moore et al. suggest three possible causes for the low prematurity rate (2% vs. 8%) in the Comprehensive Perinatal Program. We would like to postulate another possibility that was not considered in this article, that factor being the effect of nurse-midwifery care on improving outcomes.

Nurse-midwives have a long history of effecting improved perinatal outcomes.[1-9] The nurse-midwives of the Frontier Nursing Service provided care to poor, rural women in Kentucky. Most of the women who were delivered in their homes had limited resources. The Frontier Nursing Service had a maternal mortality of 1.2/1000 of 8596 births from 1925 to 1951. National maternal mortality rates were 6.73/1000 in 1931 and 3.76/1000 in 1940.[1]

Levy, Wilkinson, and Marin[3] in a 1971 study reported the impact of a nurse-midwifery program in Madera County, California.[3] During the program the prematurity rate was reduced from 11/1000 to 6.6/1000, which later rose to 9.8/1000 after discontinuance of the program. Neonatal mortality rose from 10.3/1000 during the program to 32.1/1000 after the program was discontinued. The authors related these significant changes to the type and quality of maternal/infant care provided by the nurse-midwives. During the program more prenatal care was given more frequently to a larger proportion of expectant women, and more women returned for their 6-week postpartum exami-

nations. There was a complete reversal of these trends after the program was discontinued.[3, 4]

In a 1985 study of nurse-midwifery management of adolescent pregnancy (with obstetrician consultation readily available) Piechnik and Corbett[5] reported a 50% decrease in low birth weight among adolescents under age 15, compared to the control group of adolescents cared for by state-supported maternal and child care clinics. This low birth weight rate of the nurse-midwifery group of all adolescents was 28% lower than the control group.

In 1981 Lehrman[6] reported that the average length of time of a prenatal visit with a nurse-midwife was 23.7 minutes, with a significant amount of the time spent on teaching and counseling during the visit. In the 1975 study by the National Ambulatory Medical Care Survey of office-based physicians, it was found that most prenatal visits were 10 minutes, with 32% of the visits no longer than 5 minutes and the visits including little counseling.[7] Additionally, Slome et al.[8] reported increased patient compliance in a randomized clinical trial of nurse-midwifery care compared to university house staff care.

The Institute of Medicine's 1985 report of the Committee to Study the Prevention of Low Birth Weight concluded that more reliance should be placed on nurse-midwives to provide prenatal care to groups at high risk for low birth weight. They also reported that important components of nurse-midwifery care that may prevent low birth weight include increased patient education and counseling, increased patient compliance with treatment plan and prenatal visits, and increased access to care. The benefit of these low cost interventions cannot be minimized in preventing low birth weight.[9]

Barbara L. McFarlin, C.N.M., M.S.
Suzanne Trupin, M.D.
Department of Obstetrics and Gynecology
University of Illinois College of Medicine at Urbana-Champaign
301 E. Springfield
Champaign, Illinois 61820

REFERENCES

1. Metropolitan Life Insurance Company. Quarterly Bulletin Frontier Nursing Service 1935;11:13.
2. Platt LD, Angelini DJ, Paul RH, Quilligan EJ. Nurse-midwifery in a large teaching hospital. Obstet Gynecol 1985; 66:6.
3. Levy BS, Wilkinson FS, Marine WM. Reducing neonatal mortality rate with nurse-midwives. AM J OBSTET GYNECOL 1971;109:50.
4. Montgomery TA. A case for nurse-midwives. AM J OBSTET GYNECOL 1969;105:3.
5. Piechnik SL, Corbett MA. Reducing low birth weight among socioeconomically high risk adolescent pregnancies: successful intervention with certified nurse-midwives' managed care and a multidisciplinary team. J Nurse Midwife 1985;30:2.
6. Lehrman E. Nurse-midwifery practice: a descriptive study of prenatal care. J Nurse Midwife 1981;26:1.
7. National Center for Health Statistics. Office visits by women: The National Ambulatory Medical Care Survey. Washington DC: Public Health Service, March 1980; DHHS publication no. (PHS) 80-1976. (Vital and health statistics; series 13; no. 45).
8. Slome C, Wetherbee H, Daly M, Christensen K, Meglen M, Thiede H. Effectiveness of certified nurse-midwives: a prospective evaluation study. AM J OBSTET GYNECOL 1976;124:177.
9. Institute of Medicine. Preventing low birth weight. Washington DC: National Academy Press, 1985.

Reply

To the Editors:

We appreciate the comments of McFarlin and Trupin regarding our article. Nurse-midwifery has certainly had a positive and admirable impact on prenatal care and obstetric outcome in this and other countries.

However, our study explored only the impact of prenatal care, or its absence, on pregnancy outcome. On careful reading it should be apparent that no conclusions whatsoever can be drawn with respect to what type of health professional provided the care.

Thomas R. Moore, M.D.
William Origel, M.D.
Thomas C. Key, M.D.
Robert Resnik, M.D.
Department of Reproductive Medicine
University of California, San Diego Medical Center H-813
225 Dickinson Street
San Diego, California 92103

Sonographic sign for Down syndrome

To the Editors:

A sonographic sign consisting of increased skin or soft tissue thickening at the back of the fetal neck during the second trimester was recently described as being highly specific for Down syndrome with no false positive findings (AM J OBSTET GYNECOL 1985;153:49-52). This finding could be very interesting, since it would allow a screening for all patients not undergoing chorionic biopsy or amniocentesis without arousing unuseful concern.

However, a note of caution should be employed interpreting this sign, since, at least in the third trimester, this finding does not appear to be specific for Down syndrome. In fact, we report two cases in which this sonographic sign did not correspond with Down syndrome.

The first case was a 37-week-old fetus affected by multiple malformations including a heart defect. This pregnancy ended in a stillbirth 2 weeks later, and a hydropic fetus was delivered.

The second case was a normal fetus at 38 weeks' gestation with a deflexed attitude, characterized by a thick nuchal skinfold, which was noted also in its legs when scanned tangentially. In each case the fetus showed this sonographic sign both in a sagittal and in a transverse scanning.

Furthermore, in transverse scannings abundant fetal hair may mimic this sign. Further studies are needed, at least in the third trimester, to define strict criteria for this promising sonographic sign.

Francesco Giacomello, M.D.
Department of Obstetrics and Gynecology
II University of Rome
Policlinico Umberto I
00161 Rome, Italy

Reply

To the Editors:

We appreciate very much the interest of Dr. Giacomello in our sonographic sign of increased skin or soft tissue thickening at the back of the fetal neck during the second trimester as a specific sign for Down's syndrome. Giacomello, however, reports two cases late in the third trimester in which this sonographic sign did not correspond with Down's syndrome. Our series only addresses fetuses in the second trimester between 15 and 20 weeks, and we have not looked at the accuracy of this diagnostic sign any later than 24 weeks.

We are in agreement that fetuses of the third trimester in a deflexed attitude can have a thickened nuchal fold at the back of the neck resulting from position, and this can certainly be a pitfall in looking at the fetal neck in the third trimester. Fetal hair can also be confusing, although more easily recognized as being separate from actual soft tissues.

Looking for the sonographic sign which we have described to be specific for Down's syndrome must be done cautiously, and to date, our findings only apply to fetuses of the second trimester, since our series only included second-trimester fetuses. We have not studied this sign or its specificity and sensitivity in third-trimester fetuses. Further studies are indeed needed for this purpose in order to define strict criteria for this sign outside of the second trimester.

Beryl Benacerraf, M.D.
Diagnostic Ultrasound Associates
333 Longwood Avenue
Boston, Massachusetts 02115

Abdominal pelvic pain syndrome: Fact or fiction

To the Editors:

We read with interest the article by Slocumb on the abdominal pelvic pain syndrome (AM J OBSTET GYNECOL 1984;149:536) and the subsequent correspondence by Dhont et al. (1985;151:701-2).

Our recently published study[1] of pelvic venography in women with chronic pelvic pain without obvious organic cause shows beyond doubt that pelvic varicosities are present in the majority of these women. A venogram score of 5 or more clearly distinguishes women with pelvic pain syndrome from those who have an "organic" cause or have no pelvic pain (diagnostic sensitivity, 91%, and specificity, 89%). Abdominal pressure over the "ovarian point" commonly reproduces the pain complained of, especially on the right side. Our pelvic venography studies suggest that pressure over the ovarian point occludes the ovarian vein, and we have postulated that the resultant pain is due to the back pressure on the hilum of the ovary. We consider that pelvic pain has an organic as well as a psychological component and that the "fact or fiction" part of the title of Slocumb's article is no longer a valid question.

The treatment of this condition is not so clear. All pain must have a neurophysiological component, but we find it difficult to accept that abdominal trigger points exist in women with the pelvic pain syndrome which can be anesthetized to provide long-term cure of the pain. It should be noted that Slocumb's study is uncontrolled. There is no placebo and no controls without treatment, in the design, and hence conclusions about the efficacy of trigger-point anesthesia in this group of patients must be limited. The presence of pelvic varicosities and the predominance of the ovary as the site of the pain along with the history of long-standing emotional disturbance tend to support Taylor's concept of pelvic congestion as the somatic component of a psychosomatic disorder.

There can be no doubt of the very real need to establish an effective and easily applied therapy for this disabling condition. In view of the complex nature of pain experience, it is likely that an effective treatment will only be achieved when a better understanding of the etiology of pelvic varicosities and psychological factors in pelvic pain has been acquired.

R. W. Beard
P. W. Reginald
Department of Obstetrics and Gynaecology
St. Mary's Hospital Medical School
London W2, England

S. Pearce
Psychology Department
University College, London
London WC1, England

REFERENCES

1. Beard RW, Highman JH, Pearce S, Reginald PW. Diagnosis of pelvic varicosities in women with chronic pelvic pain. Lancet 1984;2:946-9.
2. Duncan CH, Taylor HC. A psychosomatic study of pelvic congestion. AM J OBSTET GYNECOL 1952;64:1-12.

Reply

To the Editors:

The letter by Beard, Reginald, and Pearce concerning their study on pelvic pain and varicosities[1] and opinions about trigger point abdominal wall pain[2] warrants an emphatic response. The authors have described the same observed painful tissues that are noted in the paper on trigger points, but they have not made an effort to reproduce that pain documenting the tissue of origin of the pain. The so-called "ovarian points"

are identical to the ileohypogastric tissues described by many[3] but have nothing to do with the underlying ovaries.[4]

If the authors were to perform the abdominal wall test by elevating the patient's legs to tense the rectus muscles, they would see that the pain exists in the wall above the rectus. If they were to explore slowly the ovarian point in the abdominal wall fat with a No. 22 needle, they would reproduce the same pain; if they were interested in helping the patient with her pain, they could infiltrate the pain point with 3 cc of ¼% bupivacaine to block the painful tissue.

What other observations have we seen which suggest that ovaries are not the main source of this pain? (1) The same pain is found in women with and without the ovaries, broad ligaments, and/or uterus.[4] (2) Gentle pressure over the abdomen with one finger is all that is needed to reproduce the pain without any deep compression to the vessels. (3) The same pain is present with or without varicies of the ovaries.[1]

I cannot comment on the author's observations about altered blood flow to the pelvis other than to say that pain alters sympathetic return with resultant vasodilatation in the same dermatome. Of note is the fact that their observations have no clinical value at this time in terms of altering the pain.

In conclusion, I cannot stress enough the importance of a few simple modifications of the physical examination in evaluation of pelvic pain. The finding of the abdominal wall component is crucial in avoidance of unnecessary procedures if not in treating the pain itself.

John C. Slocumb, M.D.
Department of Obstetrics and Gynecology
University of New Mexico School of Medicine
2211 Lomas Boulevard, N.E.
Albuquerque, New Mexico 87131

REFERENCES

1. Beard RW, Highman JH, Pearce S, Reginald PW. Diagnosis of pelvic varicosities in women with chronic pelvic pain. Lancet 1984;2:946-9.
2. Slocumb JC. Neurological factors in chronic pelvic pain: trigger points and the abdominal pelvic pain syndrome. AM J OBSTET GYNECOL 1984;149:536-43.
3. Atlee HB. Acute and chronic iliac pain in women. Springfield, Illinois: CC Thomas, 1966.
4. Slocumb JC. Chronic pelvic pain after hysterectomy. In: Proceedings of the thirty-second annual meeting of the American College of Obstetricians and Gynecologists, San Francisco, California, May 6, 1984.

Use of midforceps and associated risk

To the Editors:

The latest item to fuel the debate over the use of midforceps and its associated risk is the article by Dierker et al. (AM J OBSTET GYNECOL 1986;154:764-8), which offers little comfort to protagonists. Although the authors deserve praise for their tempered recommendations against a return to difficult midforceps procedures, the paper may have a contrary effect on clinical practices. The fact that the major neurological sequelae were just as bad after midforceps use as after cesarean section will be interpreted by readers to indicate that midforceps delivery is safe despite the authors' disclaimer to the contrary. The report deals with infrequently done midforceps operations (0.8% over 6½ years) limited nearly exclusively to relatively "easy" procedures carried out under careful supervision after appropriate consideration of indications (and contraindications, no doubt) in highly selected circumstances, conditions not likely to be capable of duplication in very many communities. It is dubious in fact that the model—or its results—could be translated into practical reality at large.

Several points deserve special comment. First, nearly 38% of the index cases were lost to follow-up. Given this large proportion, some attempt should be made to ascertain the degree of bias this may have introduced. Pollsters now recognize that nonrespondents can no longer be ignored because the reason they do not cooperate may be related to the very issue under study. Many of the cases lost to follow-up here may therefore be those who did not turn out well. Clearly, if the lost group did contain a substantially greater frequency of the more seriously affected children, the validity of the findings would be open to question. Although the pairing design used for the study helps overcome some of this effect, it nonetheless deserves to be addressed. Second, the matching process did not include the delivery year. Because case finding extended over >6 years, it might have been prudent to consider that subtle (or perhaps overt) changes in obstetric and pediatric care practices during this interval needed to be taken into account. This turned out to be an unexpectedly important confounding factor, for example, in one of our studies of the impact of fetal heart rate monitoring.[1] Third, the time factor may also have had a further influence on the outcome findings, especially if the mean follow-up duration was shorter for the midforceps offspring than for those delivered by cesarean section. The earlier the date of the delivery, the longer the opportunity for follow-up and the greater the probability of loss to follow-up. Furthermore, the longer the follow-up, the greater the likelihood of uncovering adverse effects. Fourth, major labor disorders were collapsed under the term "dystocia" (by which we assume the authors intended to convey the concept of obstructed labor). Since only about half such cases are related to documentable cephalopelvic disproportion, it is a matter of chance that midforceps and cesarean section cases will be properly matched for this factor. In addition, it was likely for the more obviously disproportionate cases to be delivered by section, thereby further diminishing their comparability. Dystocia in the midforceps group was also far more likely to be associated with arrest of descent, whereas cesarean sections, except those undertaken in the second stage, were done more often in conjunction with protracted or arrested dilatation. Fifth, the same criticism probably applies to

matching for fetal distress. The criteria used to define it are not given in the paper. Moreover, the criteria have varied over the past decade, they are still not uniformly defined or accepted, and the diagnosis is associated with a high frequency of false positives. Reliable matching can thus be challenged. Sixth, no neurological, psychological, or intelligence testing or formal examinations were actually done for purposes of acquiring uniform and reliable information for this report, which is based instead on chart reviews and interviews. Gross deficits can probably be detected in this way, but subtle disorders are easy to miss. The more subtle deficits in speech, language, hearing, abstract and cognitive thinking, behavior, and so-called neurological soft signs are more common, more difficult to detect objectively, and take a longer follow-up time to assess adequately or to disclose definitively.

Finally, the results can just as logically be interpreted rather differently: Serious obstructive or hypoxic labor problems were shown to impact adversely on the fetus regardless of the mode of delivery, perhaps related to delays in detection, evaluation, and expeditious interventive management, and whatever additional deleterious effect the delivery may have had was not large enough to be detected by this investigative approach. This conclusion is analogous to the Scottish legal verdict of "not proved" and carries a very different meaning from that given by Dierker et al. who went too far (without objective justification, we believe) when they wrote "selected midforceps procedures may be performed with no greater risk of abnormal outcome than that associated with cesarean section," the equivalent to a "not guilty" verdict. Not proving harm is patently not the same as proving no harm. We would therefore admonish obstetricians to avoid the temptation of misinterpreting the results of this study as giving them license to turn back the clock.

Emanuel A. Friedman, M.D., Sc.D.
David B. Acker, M.D.

Department of Obstetrics and Gynecology
Harvard Medical School
Beth Israel Hospital
330 Brookline Avenue
Boston, Massachusetts 02215

REFERENCE

1. Neutra RR, Fineberg SE, Greenland S, Friedman EA. The effect of fetal monitoring on neonatal death rates. N Engl J Med 1978;299:324-6.

Reply

To the Editors:

We are pleased with Friedman and Acker's interest in our paper and appreciate the opportunity to respond to their comments. As noted by Friedman, nearly 38% of the index cases were lost to follow-up. Attempts were made to locate the family when the child's chart could not be located. The inability to locate the family therefore would not imply poor outcome for those who could not be located. Data were obtained for all subjects who were located. We do not anticipate significant bias, since the family could not be located. The situation would clearly be different if the subjects were adults without dependent family ties. The paired design was meant to minimize this effect.

The match for the midforceps group included birth weight within 200 gm, gestational age within 2 weeks, sex, race, dystocia (arrest or protraction), as well as fetal heart monitoring suggesting fetal distress. The match was made for the best subsequent match delivered by cesarean section. The follow-up for the midforceps group was therefore longer, allowing more time to uncover abnormalities. In addition, more current technology would have been available for the cesarean section group delivered later. These factors would tend to bias in favor of the cesarean group.

Friedman criticized the combination of labor disorders under the term "dystocia." Dystocia in our paper referred to arrest and protraction disorders of active and second-stage labors. The disorders were grouped, since his study (1) showed no difference between arrest or protraction disorders and intelligence quota scores—they were diminished equally. It may well be that the cesarean group contained a greater degree of cephalopelvic disproportion than the midforceps groups, but attempting to match for the degree of disproportion is not currently feasible.

As noted by Friedman, the fetal distress match was not identical. The criterion used for matching was a fetal heart rate pattern that pushed the clinician to act because of concern for fetal well-being. These patterns included late decelerations, severe variable decelerations, and bradycardias. Individual patterns and degree of severity, confirmed by scalp pH would have been ideal, but were not possible. The patterns were of sufficient concern to precipitate obstetrical intervention by either midforceps or cesarean section.

The criticism that individual formal testing was not prospectively applied was fully appreciated and mentioned in our paper. Pediatric clinic and office visits included developmental assessment of the child as an integral part of the visit. The history is designed to detect developmental delays and speech difficulties as well as health status. When abnormalities were suspected from this evaluation, children were referred for specialized analysis. The abnormalities noted in our paper were confirmed abnormalities.

Finally, in the last three paragraphs of the discussion we stated that one could not conclude the midforceps were safe. Our study was based on relatively "easy" midforceps usually from the +2 station or lower. Midforceps were not used strictly for training purposes, and continued fetal observation was encouraged. Cesarean section under those conditions was not associated with improved outcome. This is a far cry from recommending a return to difficult midforceps procedures, which we specifically note in the final paragraph of our paper.

Finally, we would note, as have Friedman et al.[1] and other authors in similar reports, that many questions regarding midforceps cannot be answered without randomized prospective studies, which are not likely to be conducted today. However, our study compared midforceps management with matched cesarean section patients, a comparison that Friedman could not do with their data. Our report suggests that altering birth route did not alter outcome. Perhaps the only way to avoid poor outcome associated with dystocia is to avoid dystocia.

LeRoy J. Dierker, Jr., M.D.
Cleveland Metropolitan General Hospital
Case Western Reserve University
Cleveland, Ohio 44109

Mortimer G. Rosen, M.D.
Columbia University
Columbia-Presbyterian Medical Center
New York, New York 10032

REFERENCE

1. Friedman EA, Sochtbeben-Murray MR, Dahrouge D, Neff RK. Long-term effects of labor and delivery on offspring: a matched-pair analysis. Am J Obstet Gynecol 1984; 150:941-5.

Kell sensitization in pregnancy

To the Editors:

I am concerned by the implications and recommendations from the article by Caine and Mueller-Heubach on Kell sensitization in pregnancy (Am J Obstet Gynecol 1986;154:85-90).

The recommendation is that when the maternal anti-Kell titer is <1:32 (Coombs technique) good care requires only monthly anti-Kell titers starting at 23 weeks. Nothing could be further from the facts. In our own experience and the experience of several others, fetal hydrops and death have been seen with titers <1:32.

The implication that titer levels from one laboratory can be compared to titer levels from another and have the same clinical implications reflects a lack of understanding of the concept of the "critical titer" level. Clarification is needed to prevent mismanagement.

Fredric D. Frigoletto, Jr., M.D.
Harvard Medical School
Maternal-Fetal Medicine
Brigham and Women's Hospital
75 Francis Street
Boston, Massachusetts 02115

Reply

To the Editors:

We appreciate the comments of Dr. Frigoletto regarding our paper on Kell sensitization in pregnancy. Our paper describes our experience with Kell sensitized pregnancies in which maternal anti-Kell titers <1:32 were not associated with significant adverse neonatal outcome. This is based on the large-scale experience of our laboratory with use of a standardized laboratory procedure for antibody titer determination. The great variation from one laboratory to another in titer determinations is well known. Therefore we never rely on antibody titers from other institutions when patients are referred to us but repeat these titers in our own laboratory. It is of interest that Dr. Frigoletto has seen fetal hydrops with a titer of 1:32 secondary to Kell sensitization. This value would have prompted us to do the full investigation outlined in our paper. Recently a case of fetal hydrops with an anti-Kell titer of only 1:4 has been reported.[1] In view of our very large series of Kell sensitized pregnancies with good outcome when the anti-Kell titer was <1:32 it appears unlikely that we were merely fortunate. It is more likely that differences are due to the variation in antibody titer determinations between laboratories which are well known and have been emphasized again by Dr. Frigoletto in his letter.

Mark E. Caine, M.D.
Eberhard Mueller-Heubach, M.D.
Department of Obstetrics and Gynecology
University of Pittsburgh School of Medicine
Magee-Womens Hospital
Forbes and Halket Street
Pittsburgh, Pennsylvania 15213

REFERENCE

1. Grannum PA, Copel JA, Plaxe SC, Scioscia AL, Hobbins JC. In utero exchange transfusion by direct intravascular injection in severe erythroblastosis fetalis. N Engl J Med 1986;314:1431.

Selection of study patients with premature rupture of membranes

To the Editors:

We read with considerable interest the report of Morales et al. (Am J Obstet Gynecol 1986;154:591-5) regarding the use of dexamethasone administration in the management of patients with preterm rupture of the membranes. This diagnosis constitutes a large segment of our maternal-fetal transport population, and insight into management strategies that will improve the outcome for mothers and infants would be extremely helpful. Unfortunately, the authors missed an opportunity to answer definitively the question of whether glucocorticoid administration is useful in the prevention of the respiratory distress syndrome in patients who have premature rupture of the membranes.

The authors state that they excluded patients in whom phosphatidylglycerol was present in the vaginal pool. Did all the *included* patients have vaginal pools assessed for the presence of phosphatidylglycerol? How was it measured? Phosphatidylglycerol assessed by Amniostat was negative in >70% of patients at Denver General Hospital whose lecithin/sphingomyelin ratios

were >2. Indeed, even in the absence of phosphatidylglycerol by any determination, the authors would have to agree that the risk of respiratory distress syndrome is considerably different with an lecithin/sphingomyelin ratio of 0.8 to 1 as opposed to a ratio of 1.8 to 1. Apparently, amniocenteses were not done in an effort to stratify treated and untreated patients by the degree of the immaturity of their lecithin/sphingomyelin ratios. This is an absolutely critical determination if one hopes to answer the question of the merits of glucocorticoids in patients with premature rupture of the membranes, since these patients represent a highly selected group of preterm gestations whose amniotic phospholipid status is significantly different from control values.

Their own data from Table III reflects this important point.

Among the 27 steroid-treated patients with a latency period of less than 24 hours, only 26% (seven patients) experienced respiratory distress syndrome as compared to 54% (35 patients) with a similar latent period and no steroid treatment. If enzyme induction of surfactant production is the mechanism by which steroids influence the occurrence of respiratory distress syndrome, certainly <24 hours is insufficient time to affect surfactant production; therefore this significant diminution from control must be explained by another reason. As the latent period increased past the "critical" 24- and 48-hour periods, no further reduction in respiratory distress syndrome is seen. This does not seem to us a biologically reasonable result. Rather, it is more easily explained by the inclusion in the steroid-treated group of some patients whose risk of respiratory distress syndrome is low; for example, a lecithin/sphingomyelin ratio of 2.2 to 1 with no phosphatidylglycerol. Though one might argue that with sufficiently large groups, the prevalence rate of very low, moderately low, and nearly mature lecithin/sphingomyelin ratios would be evenly distributed, this remains to be shown. The only study that satisfactorily addresses this problem to date is that of Garite et al.[1] in which all patients included had lecithin/sphingomyelin ratios of <2. As Morales et al. point out, that study showed no significant difference in the incidences or severity of respiratory distress syndrome in steroid or control groups. Even in that study, it would have been interesting to have patients stratified even further into very low lecithin/sphingomyelin ratios versus nearly mature lecithin/sphingomyelin ratios to note any differences associated with steroid use. It may be that the subgroups would be too small to be of any statistical usefulness, though the very low lecithin/sphingomyelin ratio subgroup are the patients for whom steroid therapy may potentially have some merit.

Even in the absence of infectious morbidity in the current study, it should be noted that tocolytic therapy was required in 44 patients, including two patients who had acute pulmonary edema. The mere absence of phosphatidylglycerol from a vaginal pool is unsatisfactory information in our view to prolong a pregnancy with tocolysis in the face of ruptured membranes in order to administer steroids. Additional information regarding the true risk of respiratory distress syndrome in these infants as well as ruling out subclinical infection by amniocentesis is necessary to plan intelligently management strategies for these high-risk pregnancies.

Richard P. Porreco, M.D.

Maternal-Fetal Medicine
Reproductive Genetics
St. Luke's/Denver Children's Hospital
Perinatal Program
Denver, Colorado 80203

M. Shannon Burke, M.D.

Maternal-Fetal Medicine
Denver General Hospital
Denver, Colorado 80204

REFERENCE

1. Garite TJ, Freeman RK, Linzey EM, Braly PS, Dorchester WL. Prospective randomized study of corticosteroids in the management of premature rupture of the membranes and the premature gestation. AM J OBSTET GYNECOL 1981; 141:508-15.

Reply

To the Editors:

We appreciate the interest of Porreco and Burke in our work and thank them for their comments.

Phosphatidylglycerol was determined from samples obtained from vaginal pool by thin-layer chromatography, although since 1985 it is being assessed by Amniostat. This was generally determined in the more mature preterm gestations of ≥32 weeks. While we do agree that the risk of respiratory distress syndrome is decreased by increased lecithin-sphingomyelin ratio, the preterm infant of <32 weeks is at risk of other serious complications such as intraventricular hemorrhage and retinopathy of prematurity; although their incidences are increased by the severity of their respiratory distress, mild respiratory distress syndrome could still result in significant rates. Nonetheless, we are currently evaluating as a part of a study investigating other treatment modalities, data on the effects of dexamethasone on preterm pregnancies with different lecithin-sphingomyelin ratios. In regard to the results of Table III, latency period in this study was defined (as noted in the text) as the time from rupture of membranes to the onset of labor, *not* to delivery. Although 27 patients in the steroid treatment group had a latency period of <24 hours, 22 (81%) completed their course of therapy by the complementary use of intravenous tocolytic agents.

Walter J. Morales, M.D., Ph.D.
Division of Maternal and Fetal Medicine
Department of Obstetrics and Gynecology
1 Davis Boulevard, Suite 310
Tampa, Florida 33606

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

Reproductive Endocrinology and General Gynecology, May 31–June 6, 1987, Palmetto Dunes Resort, Hilton Head Island, South Carolina. Sponsored by the Department of Gynecology and Obstetrics, The Johns Hopkins Medical Institutions. For information contact: Jeanne Ryan, Program Coordinator, Office of Continuing Education, The Johns Hopkins Medical Institutions, 720 Rutland Ave., Turner 22, Baltimore, MD 21205. Tel.: (301) 955-6046.

High-Risk Obstetrics, April 2-4, 1987, Chicago, Illinois. Sponsored by the Cook County Graduate School of Medicine. For additional information contact the Registrar's Office. Toll-free number: Illinois 1 (800) 621-4649; outside Illinois 1 (800) 621-4651.

The First International Symposium on Intrapartum Biochemical Monitoring of the Fetus, June 1-3, 1987, Caesar's Casino and Hotel, Atlantic City, New Jersey. Contact: M. Chatterjee, M.D., Our Lady of Mercy Medical Center, 600 East 233rd St., Bronx, NY 10466. Tel.: (212) 920-9647.

Specialty Review in Obstetrics and Gynecology, April 20-25, 1987, Chicago, Illinois. Sponsored by the Cook County Graduate School of Medicine. For additional information, contact the Registrar's Office. Toll-free number: Illinois 1 (800) 621-4649; outside Illinois 1 (800) 621-4651.

A Forum on Reproductive Laws for the 1990s, Monday, May 4, 1987, The Doral Inn, 50th Street and Lexington Avenue, New York, NY 10022. Tel.: (212) 755-1200. For further information contact: Women's Rights Litigation Clinic, Rutger's University School of Law, 15 Washington St., Newark, NJ 07102.

The Pacific Coast Obstetrical and Gynecological Society Ted Adams Scholarship Committee invites applications for an award of $7,500 to be given in 1987 to a student, resident, or practicing physician residing in the geographical area of the society (Alaska, Arizona, British Columbia, California, Hawaii, Idaho, Nevada, Oregon, Utah, and Washington). Applicants should submit a descriptive outline detailing their educational background, their present status, and how the award would be used in pursuit of knowledge that could elucidate and contribute to understanding of some aspect of normal or abnormal structural, functional, or behavioral component of human reproductive biology. All applications received by July 1, 1987, will be considered by the Committee. The decision of the Committee will be final and will be announced at the annual meeting of the Society, September 27 to October 2, 1987, at the Lodge at Pebble Beach, Pebble Beach, California. Address applications to: PCOGS Scholarship Committee, 707 Cobb Medical Center, Seattle, WA 98101.

Ninth Practical Course in Colposcopy and Cervicovaginal Histopathology in English. Demonstration of the Use of Dr. Cartier's Diathermy Loop, Paris, France, September 21-25, 1987. For further information contact: Dr. R. Cartier, 20, rue des Cordelieres, F 75013 Paris, France. Tel.: (1) 43 36 04 46.

The Mt. Sinai Medical Center of Cleveland Gynecologic and Infertility Laser Surgery Workshop, May 11-13, 1987, The Mt. Sinai Medical Center, Cleveland, Ohio. To obtain further information, write to: Dr. Robert Kiwi, The Mt. Sinai Medical Center, One Mt. Sinai Dr., Cleveland, OH 44106. Tel.: (216) 421-4607.

Chicago Area Schools of Medicine Obstetrics and Gynecology Review Course, June 8-13, 1987, Americana Congress Hotel, Chicago, Illinois. For information contact: The University of Chicago, Office of Continuing Medical Education, 5841 S. Maryland, Box 139, Chicago, IL 60637. Tel.: (312) 702-1056.

National Abortion Federation wishes to announce its Eleventh Annual Meeting, "Abortion Services: Contributions to the Nation's Health," May 17-20, 1987, Salt Lake City Marriott Hotel, Salt Lake City, Utah. For more information, please contact NAF at (202) 546-9060.

ASA/1/87

UNIVERSITY OF ZIMBABWE

APPLICATIONS ARE INVITED FOR THE FOLLOWING POSTS:
1. LECTURESHIPS/SENIOR LECTURESHIPS/ASSOCIATE PROFESSORSHIPS:

DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY (2 Posts)
Applicants must hold the MRCOG or equivalent higher qualification plus relevant experience reflected by substantial publications in reputable journals. This is a senior post requiring candidates to undertake undergraduate and postgraduate teaching, research and clinical services.

SALARY SCALE
(Medical)
(Including Professional Supplement)

Lecturer	: Z$22 224 - Z$30 840
Senior Lecturer	: Z$31 740 - Z$33 960
Associate Professor	: Z$34 884 - Z$35 640

Appointments on the above scales according to qualifications and experience.

CONDITIONS OF SERVICE
Both permanent and short-term contracts are offered. Persons who are not Zimbabwean citizens may be appointed only on a short-term contract basis for an initial period of two years. Short-term contracts may be extended.

To apply, send six copies of resume and curriculum vitae, which should include full name, place and date of birth, qualifications, employment and experience, present salary, date of availability, telephone number and names and addresses of three referees should be addressed to the:

Director, Appointments and Personnel
University of Zimbabwe
P O Box MP 167
MOUNT PLEASANT
Harare
Telegrams: UNIVERSITY
Telex: 4-152 ZW

Applicants may obtain conditions of employment and job description from Mr. S. W Cole, (Vice-President), African American Institute, 833 United Nations Plaza, New York, 10017 U.S.A. but application material must be sent directly to the University of Zimbabwe as instructed above.
Closing date for receipt of the applications is 1, April, 1987.

OBSTETRICIAN-GYNECOLOGIST

Obstetrician-Gynecologist needed to join seven person Department in 165 multi-specialty clinic; clinic and adjacent 288-bed hospital are located in Big 10 University community of 100,000; teaching positions are available in medical school for those with academic inclinations; liberal fringe benefits and salary lead to equal ownership. Write including CV to:

Executive Vice-Chairman
Carle Clinic
Urbana, IL 61801

DIRECTOR
Obstetrics & Gynecology

Coney Island Hospital, a large modern teaching hospital located in a very desirable location, in a pleasant and highly supportive residential community, is currently offering a position as Director to head the Department of OB/GYN.

Our Department is professionally affiliated with Maimonides Medical Center through which it maintains an affiliation with State University of New York-Medical Campus at Brooklyn.

Candidate should be board-certified, aggressive, academically-oriented, with a strong interest and experience in Gynecology.

Participation in the fee-for-service practice of CIMG, P.C. offers an exceptional compensation package. Faculty appointment at rank commensurate with training and experience.

Qualified applicants should forward C.V. which will be held in strictest confidence to:

BURTON HERZ, M.D., CHAIRMAN SEARCH COMMITTEE
CONEY ISLAND HOSPITAL
2601 OCEAN PARKWAY, HAMMET PAVILION RM 303, BROOKLYN, N.Y. 11235

An Equal Opportunity Employer M/F

OB/GYN
OREGON

Ob/Gyn BE/BC wanted to join four BC Physicians in a well established practice in great college community.

Excellent benefits include paid malpractice insurance, pension, retirement benefits and partnership opportunity.

Please send cv and references to:

CORVALLIS OBSTETRICS & GYNECOLOGY, P.C.
3640 NW Samaritan Drive, Suite 270
Corvallis, Oregon 97330
(503) 758-3778

HAHNEMANN UNIVERSITY
DEPARTMENT OF OBSTETRICS AND GYNECOLOGY

Immediate Openings Available For

Gynecologic Oncologist
Reproductive Endocrinologist
Obstetric & Gynecologic Generalist

Applicant must be Board Eligible or Certified and certifiable by subspecialty board when applicable.

Salary and Rank Negotiable
Based on Experience and Qualifications

Interested individuals should contact:

Michael R. Spence, MD, Chairman
Department of Obstetrics and Gynecology
Hahnemann University Hospital
Broad and Vine Streets
Philadelphia, PA 19102

PHYSICIAN

DIRECTOR, OB/GYN

Jersey City Medical Center is a major acute care teaching hospital in Northern New Jersey with an approved residency program in Obstetrics & Gynecology. Board certification required. May have Boards in sub-specialty area; perinatology a plus (this Medical Center services as a regional perinatal center, with a neonatal intensive care unit & experienced neonatal staff). The program is building, and searching for a creative clinician & educator. Teaching experience with clinical orientation required. Opportunity to build faculty practice.

We offer a competitive salary & comprehensive benefit package. Please reply in writing to **Richard Lauterhahn, Personnel Recruiter.**

JERSEY CITY MEDICAL CENTER
50 Baldwin Avenue
Jersey City, NJ 07304

Maternal-Fetal Medicine

Board certified/eligible PERINATOLOGISTS required for large volume obstetric services (3,000-6,000 deliveries per year).

Opportunities exist both to join and create high risk obstetric services at various Kaiser Permanente Medical Care Centers located in Southern California. University affiliations and clinical research is encouraged.

Excellent benefits are offered including liability coverage, medical and dental coverage, vacation and sick leave, continuing education and retirement program. Partnership consideration available after two years full time employment.

Please write, including your curriculum vitae and three professional references to:

KAISER PERMANENTE
SOUTHERN CALIFORNIA
PERMANENTE MEDICAL GROUP

William G. McCormick M.D.
Physician Recruitment
Dept. 41B
Walnut Center
Pasadena, CA 91188
(714)829-5365
Equal Opportunity Employer M/F/H

OB-GYN

EXCELLENT OPPORTUNITY FOR BOARD CERTIFIED or eligible OB/GYN Physician to join twenty-nine physician, well-established, multi-specialty group. Will join two established Obstetricians. Good starting salary with an incentive program. Excellent corporate fringe benefit program. Practice situated in medium size city 45 miles north of Denver, Colorado. Contact:

Denise M. Miller, M.D.
c/o Longmont Clinic, P.C.
1925 Mountain View Ave.
Longmont, Colorado 80501

FACULTY POSITIONS MATERNAL FETAL-MEDICINE

The Department of Obstetrics and Gynecology at the Medical College of Ohio at Toledo, is accepting applications for full time faculty positions within the Division of Maternal Fetal Medicine. The Center for Women and Children is the level III Perinatal Referral Center for Northwest Ohio and has over 4400 deliveries yearly. There is a 60 bed NICU with 6 full time neonatologists. The candidate should be board certified or board eligible in Maternal Fetal Medicine. Duties will include patient care, teaching activities with residents and medical students and development of research activities. Salary and academic rank will be commensurate with the candidate's training and experience. Please direct inquiries and curriculum vitae to:

Roger R. Lenke, M.D.
Associate Professor
Director Maternal Fetal Medicine
Medical College of Ohio
C.S. 10008
Toledo, Ohio 43699
419-381-4591

Michael Reese Health Plan

A LARGE HOSPITAL-BASED HMO

has openings for the following full-time position:

Obstetrician Gynecologist

THE MICHAEL REESE HEALTH PLAN, a well-established and rapidly-expanding, federally-qualified HMO seeks Board Certified/Eligible Obstetrician/Gynecologists.

THIS POSITION entails both outpatient and hospital practice as well as medical students and house staff teaching.

REMUNERATION is high and competitive with academic and fee-for-service practice markets. Fringe benefits are generous.

INTERESTED APPLICANTS should send Curriculum Vitae in confidence to:
Dr. Stephen Contro, Medical Director,
MICHAEL REESE HEALTH PLAN,
2545 Martin Luther King Drive,
Chicago 60616

an equal opportunity employer m/f/h

FACULTY POSITION
DEPARTMENTS OF OB/GYN AND MEDICAL GENETICS
THE OREGON HEALTH SCIENCES UNIVERSITY

Opening at junior faculty level to work under the direction of the program director in prenatal diagnosis.

M.D. certified or eligible in American boards of Medical Genetics, Obstetrics and Gynecology and preferably in subspecialty board of Maternal-Fetal Medicine.

Responsibilities include patient service in prenatal diagnosis, in-house and outreach education, clinical or basic research opportunities, new program development and some high-risk obstetrical consultation.

Please send curriculum vitae to:

John Bissonnette, M.D.
The Oregon Health Sciences University
3181 S.W. Sam Jackson Park Road, L-458
Portland, OR 97201

The Oregon Health Sciences University is an Equal Opportunity Affirmative Action Employer

FACULTY POSITION AVAILABLE

The Department of Obstetrics and Gynecology at the Medical College of Ohio is accepting applications for a full-time position as director of the residency training program and generalist in the field of Obstetrics and Gynecology.

The residency director is responsible for administration of the program and patient care, including a busy clinical service along with resident and student teaching. Salary and academic rank will be commensurate with candidate's training and experience. Please direct inquiries and curricula vitae to:

John L. Duhring, M.D.
Chairman and Professor
Department of Obstetrics-Gynecology
Medical College of Ohio
C.S. 10008
Toledo, Ohio 43699
(419) 381-4591

MEDICAL-RESEARCHER

Duties consist of doing biochemical research projects and writing papers. Observes surgery and patient care in connection with medical research. No actual patient care. 2 yrs. exp. reqd. or 3 yrs. as Obstetrician/Gynecologist; 40 hr/wk; 9-5; $27,600 per annum. Resume to: Bronx Job Service, 349 East 149th Street, Bronx, N.Y. 10451. JO# 8004229, DOT 070.101.022

OB/GYN CHAIRPERSON

Monmouth medical center, a 526 bed major teaching affiliate of Hahnemann University School of Medicine seeks Chairperson for Department of Ob/Gyn starting July 1, 1987. 9 fully accredited residency programs including Ob/Gyn 3rd yr medical Student clerkship. Qualifications: Board certification; clinical, administrative, teaching & research experience; academic credentials for appointment as associate professor or professor. Full Time salary plus limited practice. Reply with C.V. to:

Chairman,
Ob/Gyn Search Committee
Medical Staff Office

Monmouth Medical Center
300 Second Avenue
Long Branch, New Jersey 07740
Equal Opportunity Employer M/F

OBSTETRICIAN/ GYNECOLOGIST

The Smith-Glynn-Callaway Clinic, Inc., located in Springfield, Missouri, is seeking a physician university trained, board eligible or certified in obstetrics/gynecology to join its 66-physician, multispecialty group practice serving a regional population area of 1,000,000+. Competitive salary with bonus/production incentive. Fringe benefits package includes life, health, pension plan, vacation, paid meeting expenses. Four hospitals with combined bed capacity of 1,766. Excellent secondary schools, four universities. Please call or write to Administrator, 3231 South National, Springfield, MO. 65807 or call collect 417-883-7422.

MATERNAL-FETAL MEDICINE
(Board eligible or Certified)

600 Bed University-affiliated Level III Hospital with 3,000 deliveries/year, 24-hour in-house anesthesiology and neonatology/large NICU, active maternal transfer service, perinatal outreach programs, and fully-approved OB/GYN residency program has immediate opening for a full-time position in perinatology. Duties to include residency teaching, maternal transfer care, and private practice incentive. Competitive guaranteed salary plus incentive. Excellent public school system, housing, and Lake St. Clair in very close proximity.

CALL COLLECT: 313-343-3495
SAINT JOHN HOSPITAL
DEPT. OB/GYN
DETROIT, MI 48236

Subspecialist Positions

615-bed tertiary care facility is seeking several board eligible or board certified subspecialists for the following areas within an expanding Department of Obstetrics and Gynecology.

- **Maternal/Fetal Medicine**
  Two new positions available in our established division of Maternal/Fetal Medicine. Ultrasound experience desirable.
- **Reproductive Endocrinology**
  An exceptional growth opportunity is available for a physician to direct this new division.

These positions offer excellent opportunities for professional development to qualified physicians interested in subspecialty practice, resident training, and research/faculty status in a large university affiliated hospital.

SINAI HOSPITAL OF DETROIT

Please submit your Curriculum Vitae or contact **Dr. David B. Schwartz, Chairman, Department of Obstetrics and Gynecology, Sinai Hospital of Detroit, 6767 W. Outer Drive, Detroit, MI 48235, or call (313) 493-5220.**

MATERNAL-FETAL MEDICINE SPECIALIST

Excellent opportunity for a BC/BE Perinatologist to join an expanding Division of Perinatology. The position provides an opportunity for resident and student supervision and teaching, access to an established, large private practice, and clinical research. Extensive ultrasound, genetics, and maternal transport practice developed. Excellent compensation, benefits, and professional opportunities. Please send CV to:

Jorge D. Blanco, M.D.
Director of Perinatology
Memorial Medical Center
P. O. Box 23089
Savannah, Georgia 31403
OR CALL 912/355-8522

MATERNAL-FETAL MEDICINE
(Board Eligible or Certified)

1000 Bed University-affiliated, Level III Hospital with 6,000 deliveries/year. Fully-approved OB/GYN residency program. 24-hour in-house anesthesiology and neonatology/large NICU, active maternal transfer service, perinatal outreach program. Opening for a full-time position in perinatology. Training and experience in ultrasound required. Eligible for academic appointment at Thomas Jefferson University. Responsibilities include resident and medical student teaching, maternal-transfer care, and private practice. Competitive guaranteed salary with excellent fringe benefits including professional liability plus incentive income. Conveniently located in close proximity to major metropolitan areas. Interested applicants may call collect or send curriculum vitae to Dr. Garrett H.C. Colmorgen, Director, Maternal-Fetal Medicine.

CALL COLLECT: 302-733-3350
The Medical Center of Delaware
Christiana Hospital
Department of OB/GYN
P.O. Box 6001
Newark, DE 19718

Faculty Position
GEORGIA

Chief, OB/GYN in Community Hospital. Academic setting teaching Family Practice Residents who staff busy OB/GYN service in tertiary perinatal center. Emory University and Medical College of Georgia affiliate. Relaxed professional environment.

Send C.V. to:

Howard G. Vigrass, M.D.
710 Center Street
Box #84
Columbus, GA 31994

Full Time
Faculty Position
Maternal-Fetal Medicine

The department of OB/GYN at Temple University Health Science Center is seeking a Board Certified/Board Eligible Perinatologist. Clinical and academic activities are integrated in a department providing tertiary services, including genetic counseling. Newly opened Temple University Hospital houses a modern Labor & Delivery suite and large Neonatal Intensive Care unit. Approved Fellowship program. Candidates should have an active interest in Research, Clinical Practice, and Resident and Medical student education. Salary and academic rank commensurate with experience. Opportunity for qualified person to become Section Chief. Submit C.V. to:

P. Hanjani, M.D., Professor, OB/GYN

Temple University
Health Science Center
3401 N. Broad St.
Philadelphia, PA 19140

TEMPLE UNIVERSITY

An equal opportunity,
affirmative action employer

OB/GYN

MALE/FEMALE BOARD ELIGIBLE/CERTIFIED, U. S. Trained for quality practice in community of approximately 23,000. Well equipped hospital with 24 other physicians. Area features hunting, fishing camping and skiing. Close proximity to Pueblo, Colorado Springs and Denver. CALL OR WRITE: St. Thomas More Hospital and Progressive Care Center, 1019 Sheridan Street, Canon City, Co 81212, Sister M. Judith Kuhn, OSB, Chief Executive Officer. (303) 275-3381.

PACIFIC NORTHWEST

Beautiful country setting

Easy access to urban amenities

Eight person group seeks second OB/GYN for rapidly growing, low-risk practice.

Family oriented community near major urban area and just 60 minutes from outstanding ski resort.

For information call or send CV to:

The Friedrich Group, Inc.
9284 Ferncliff Northeast
Bainbridge Island, WA 98110
(206) 547-7850
(206) 842-5248

OBSTETRICS-GYNECOLOGY

POSITION AVAILABLE IN THE OB-GYN DEPARTMENT OF A 100+ PHYSICIAN MULTISPECIALTY GROUP WITH A WELL ESTABLISHED SATELLITE SYSTEM. 300,000 PATIENT POPULATION BASE. PRACTICE STATE OF THE ART MEDICINE IN A RELATIVELY RURAL AREA WITH NEARBY NATIONAL RECREATION AREA. LARGE CITIES READILY ACCESSIBLE BY PARKWAY. WELL EQUIPPED 400-BED HOSPITAL WITH NEONATAL NURSERY AND FULL RANGE OF ANCILLARY SERVICES. ALL MEDICAL AND SURGICAL SUBSPECIALTIES REPRESENTED ON STAFF. GUARANTEED MINIMUM OF $100,000 ANNUAL SALARY BASE WITH EARLY PARTICIPATION IN THE CLINIC'S PRIMARILY INCENTIVE BASED INCOME DISTRIBUTION SYSTEM. PLEASE SEND CURRICULUM VITAE TO:

Assistant Medical Director
Trover Clinic
Clinic Drive
Madisonville, Kentucky 42431
or
Call Collect: 502-825-7470

CHAIRMAN
DEPARTMENT OF OBSTETRICS AND GYNECOLOGY

The University of South Florida College of Medicine is seeking applications for Chairman. The department is a tertiary referral center for Maternal-Fetal Medicine, Gynecologic Oncology, Gynecology and Reproductive Endocrinology. The department is responsible for a large clinical and teaching program.

Applicant should be Board Certified in Obstetrics and Gynecology and have clinical, teaching and administrative experience.

Send letters of interest or nominations with CV to:

Lewis A. Barness, M.D.
Chairman, Search Committee
Chairman of OB/GYN, Box 15
USF College of Medicine
12901 Bruce B. Downs Blvd.
Tampa, FL 33612
(813) 974-4214

Deadline for application is May 31, 1987.

USF is an equal opportunity affirmative action employer.

FACULTY OPENINGS IN OBSTETRICS AND GYNECOLOGY

1. Perinatology
2. Gynecologic Endocrinology
3. General Obstetrics/Gynecology
4. Family Planning
5. Gynecologic Oncology

Rank and salary: negotiable, commensurate with training and experience.

Qualifications: M.D. with eligibility for certification by American Board of Obstetrics and Gynecology and, where applicable, certification (or eligibility for) in subspecialty is desirable. Interest and experience in undergraduate and graduate education, clinical and/or basic research, and clinical practice of obstetrics and gynecology.

For further information, please contact:

William J. Dignam, M.D.
Acting Chairman
Dept. Obstetrics & Gynecology
UCLA School of Medicine
Los Angeles, California 90024

Affirmative Action/Equal Opportunity Employer

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
Obstetrics, Gynecology and Reproductive Sciences
Assistant Professor Level
Two Positions

The University of California, San Francisco, Department of Obstetrics, Gynecology and Reproductive Sciences, is seeking two full time faculty members at the Assistant Professor level.

Obstetrician-Gynecologist
Gynecolgoic Urology

Applicants for this gynecologic-urology position should be board certified or eligible in obstetrics and gynecology with demonstrated experience in both general gynecology and gynecologic urology, capable of mounting an independent research program, as well as being well prepared clinically.

Send a curriculum vitae and bibliography to:

Richard L. Sweet, M.D.
Professor and Vice Chairman
Department of Obstetrics, Gynecology and Reproductive Sciences
San Francisco General Hospital
1001 Potrero Avenue, Room 6J-2
San Francisco, CA 94110

Obstetrician-Gynecologist
Reproductive Endocrinologist

Applicants for this obstetrician-gynecologist/reproductive endocrinology position should be board certified or eligible in reproductive endocrinology with demonstrated competence in both general obstetrics and gynecology as well as in endocrinology and infertility with demonstrated abilities and interest in clinical investigation and teaching. This position will serve approximately half time with the general clinical practice and the other half time assisting in the in vitro fertilization program.

Send a curriculum vitae and bibliography to:

Mary C. Martin, M.D.
Assistant Professor
Department of Obstetrics, Gynecology and Reproductive Sciences
University of California, San Francisco
San Francisco, CA 94143-0132

UCSF is an Equal Opportunity/Affirmative Action Employer. Women and minorities are encouraged to apply.

INDEX TO ADVERTISERS

Abbott Laboratories
Ogen 44A,45A,46A
Ayerst Laboratories
Premarin 20A,21A,22A
Berlex Laboratories, Inc.
Levlen 23A,24A
Tri-Levlen 62A,63A,64A
Carle Clinic
Opportunity Available 65A
Ciba Pharmaceutical Company
Estraderm 37A,38A,39A,40A
Coney Island Hospital
Opportunity Available 65A
Corvallis Obstetrics & Gynecology, P.C.
Opportunity Available 66A
Filmtec
Informed Consent Systems 43A
Friedrich Group, Inc., The
Opportunity Available 51A,70A
Hahnemann University
Opportunity Available 66A
Jersey City Medical Center
Opportunity Available 66A
Kaiser Permanente
Opportunity Available 66A
Life-Tech, Inc.
Equipment 18A
Longmont Clinic, P.C.
Opportunity Available 67A
Mead Johnson Laboratories
Estrace 58A,59A,60A
Natalins RX 15A
Ovcon-35 ..... 48A,49A,50A
Ovcon-50 ..... 30A,31A,32A
Medical Center of Delaware
Opportunity Available 69A
Medical College of Ohio
Opportunity Available 67A,68A
Medical Equipment Designs, Inc.
HemoCue 12A,13A
Memorial Medical Center
Opportunity Available 69A
Merck Sharp & Dohme
Mefoxin 34A,35A,36A
Michael Reese Health Plan
Opportunity Available 67A
Miles Pharmaceuticals
Mycelex Twin Pack 7A,8A
Monmouth Medical Center
Opportunity Available 68A
Mosby Company, The C.V.
Book 47A
Multigon Industries, Inc.
Doppler 10A
Oregon Health Sciences University
Opportunity Available 67A
Ortho Pharmaceutical Corporation
Ortho-Novum 7/7/7 . Third Cover, Fourth Cover
Permanente Medical Group, Inc., The
Opportunity Available 51A
Saint John Hospital
Opportunity Available 69A
Sinai Hospital of Detroit
Opportunity Available 69A
Smith-Glynn-Callaway Clinic, Inc.
Opportunity Available 68A
Smith Kline & French Laboratories, Inc.
Cefizox 52A,53A,54A
St. Thomas More Hospital
Opportunity Available 70A
Stuart Pharmaceuticals
Cefotan 41A,42A
Syntex Laboratories, Inc.
Femstat 55A,56A
Tri-Norinyl ..... Second Cover,1A,2A
Temple University Health Science Center
Opportunity Available 70A
Thomas, Charles C., Publisher
Book 43A
Trover Clinic
Opportunity Available 71A
UCLA School of Medicine
Opportunity Available 71A
University of Alabama at Birmingham
Opportunity Available 61A
University of California, Davis, Medical Center
Opportunity Available 43A,61A
University of California, San Francisco
Opportunity Available 71A
University of Geneva
Opportunity Available 57A
University of Iowa
Opportunity Available 43A
University of Minnesota
Opportunity Available 57A
University of Montreal
Opportunity Available 51A
University of South Florida College of Medicine
Opportunity Available 71A
University of Tennessee
Opportunity Available 51A,57A,61A
University of Zimbabwe
Opportunity Available 65A
Upjohn Company, The
Provera 3A,4A
WalthamWeston Hospital & Medical Center
Opportunity Available 57A
Wenatchee Valley Clinic
Opportunity Available 61A

While every precaution is taken to insure accuracy, we cannot guarantee against the possibility of an occasional change or omission in the preparation of this index.

ORTHO-NOVUM 1/77

TRADEMARK

Tablets

(norethindrone/ethinyl estradiol)

Enhanced patient compliance

Each white tablet contains 0.5 mg norethindrone and 0.035 mg ethinyl estradiol. Each light peach tablet contains 0.75 mg norethindrone and 0.035 mg ethinyl estradiol. Each peach tablet contains 1 mg norethindrone and 0.035 mg ethinyl estradiol. Each green tablet in 28-day regimen contains inert ingredients.

IMPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the product and therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables, and references. The physician should be thoroughly familiar with the complete prescribing information before prescribing the product.

INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

DOSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing .05 mg or less of estrogen.

CONTRAINDICATIONS: Oral contraceptives should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed, abnormal genital bleeding. 7. Oral contraceptive tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who are pregnant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study conducted in the United States found that past use of oral contraceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk may be related to duration of oral contraceptive use. **MYOCARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists following discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the past use. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is discontinued. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. OCULAR LESIONS. There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. CARCINOMA. Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. HEPATIC TUMORS. Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5. USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING. The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. GALLBLADDER DISEASE. Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. HEADACHE. The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. BLEEDING IRREGULARITIES. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. BREAST FEEDING. Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **INFORMATION FOR THE PATIENT: (See Patient Package Insert). DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **CARCINOGENESIS, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS and WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea, usually the most common adverse reaction. Vomiting, occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Revised 12/84

ORTHO-PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869

OJ-783

ORTHO

for new start patie

#1 F

What to look for in a phasic†...Reason 3

Enhanced patient compliance



Dial turns in only one direction to help assure accurate phasic sequence



Only one tablet—*the right tablet*—can be removed at a time

- The Ortho DIALPAK* Tablet Dispenser advantage
- Conveniently prelabeled with days of the week

7 7 7

Designed for performance

21- and 28-day regimens

ORTHO-NOVUM 7/7/7 Tablets

TRADEMARK

(norethindrone/ethinyl estradiol)

†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

Please see complete Prescribing Information, a summary of which appears on the preceding page.

ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869

OJ-783

© OPC 1985

ORTHO

11.12.90

August 1987
volume 157, *number* 2

American Journal of OBSTETRICS AND GYNECOLOGY

Copyright © 1987 by The C. V. Mosby Company

Editor in Chief
JOHN I. BREWER

Editors
FREDERICK P. ZUSPAN • E. J. QUILLIGAN

Associate Editor
ALBERT B. GERBIE

Official Publication

AMERICAN GYNECOLOGICAL AND OBSTETRICAL SOCIETY
CENTRAL ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
SOCIETY OF OBSTETRICIANS AND GYNAECOLOGISTS OF CANADA
SOUTH ATLANTIC ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
PACIFIC COAST OBSTETRICAL AND GYNECOLOGICAL SOCIETY
AMERICAN BOARD OF OBSTETRICS AND GYNECOLOGY
SOCIETY FOR GYNECOLOGIC INVESTIGATION
SOCIETY OF PERINATAL OBSTETRICIANS

Published by

THE C. V. MOSBY COMPANY
St. Louis, Missouri 63146

ISSN 0002-9378

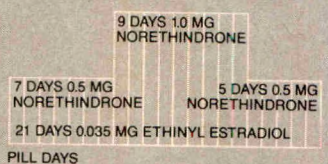


UNIQUE

Full-cycle endometrial support, with no late-cycle progestin increase.



The unique TRI-NORINYL phasing achieves BTB control equivalent to a 1/35 formulation, but with 29% less progestin per cycle. This is, in fact, the greatest progestin reduction of any norethindrone-based multiphasic. TRI-NORINYL lowers progestin where requirements for endometrial support are reduced–with no greater reported BTB than a 1/35 formulation.*



SUPPLIED IN THE EXCLUSIVE SYNTEX WALLETTE™ PILL DISPENSER



Preferred by patients 3 to 1 over other available packages for convenience, discreetness and compliance.**

THE NATURE OF TRI-NORINYL®

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

*1/35 formulations contain 1.0 mg norethindrone with 0.035 mg ethinyl estradiol. Data available from Syntex Laboratories, Inc.

**Based on a nationwide, independently conducted survey of prospective oral contraceptive patients. Data available from Syntex Laboratories, Inc.

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see brief summary of full prescribing information on next page.

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

©1986 Syntex Laboratories, Inc.

THE NATURE OF TRI-NORINYL®

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

TRI-NORINYL® 21-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)
TRI-NORINYL® 28-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg; 7 inert tablets)

ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications Prevention of pregnancy. *DOSE-RELATED RISK OF THROMBOEMBOLISM.* Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested the persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34 – 5/100,000; ages 35-44 – 33/100,000; ages 45-49 – 140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34 – 1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over – 1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only; smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and for MI is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS – RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40+
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A – Use associated with very high risk.
B – Use associated with high risk.
C – Use associated with moderate risk.
D – Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden, or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to the registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer in OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer, and other studies suggested an increased risk of breast cancer with long-term OC use before age 25. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared to nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. Two other studies suggested an increasing incidence of cervical neoplasms with longer duration of OC use. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OCs with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones – estrogenic and progestational agents – during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase the risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in the results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. One study suggests *Chlamydia trachomatis* infection, the leading cause of nongonococcal P.I.D., is enhanced by OCs. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failure. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions** *GENERAL* 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding from interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, antimigraine preparations, antihistamines, and griseofulvin. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, theophylline, and beta blockers. Toxic liver effects have been reported with a combination of an OC and cyclosporin A. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Warnings. **Adverse Reactions** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps, colitis), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, changes in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution of lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chilblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, vaginitis, porphyria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome), malignant hypertension, acute renal failure. **Information for the Patient** (See Patient Package Insert).

Rev. 6/86

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

A Standard in Progesterone Therapy

Provera® 10 MG TABLETS
(medroxyprogesterone acetate)

WARNING

THE USE OF PROGESTATIONAL AGENTS DURING THE FIRST FOUR MONTHS OF PREGNANCY IS NOT RECOMMENDED.

Progestational agents have been used beginning with the first trimester of pregnancy in an attempt to prevent habitual abortion or treat threatened abortion. There is no adequate evidence that such use is effective and there is evidence of potential harm to the fetus when such drugs are given during the first four months of pregnancy. Furthermore, in the vast majority of women, the cause of abortion is a defective ovum, which progestational agents could not be expected to influence. In addition, the use of progestational agents, with their uterine-relaxant properties, in patients with fertilized defective ova may cause a delay in spontaneous abortion. Therefore, the use of such drugs during the first four months of pregnancy is not recommended.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies including congenital heart defects and limb reduction defects. One study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000.

If the patient is exposed to PROVERA Tablets (medroxyprogesterone acetate) during the first four months of pregnancy or if she becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus.

INDICATIONS: Secondary amenorrhea; abnormal uterine bleeding due to hormonal imbalance in the absence of organic pathology such as fibroids or uterine cancer.

CONTRAINDICATIONS: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy or patients with a past history of these conditions. Liver dysfunction or disease. Known or suspected malignancy of breast or genital organs. Undiagnosed vaginal bleeding. Missed abortion. Known sensitivity to medroxyprogesterone acetate. As a diagnostic test to pregnancy.

WARNINGS: 1. Immediately discontinue administration should any of the following thrombotic disorders occur or be suspected: thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis. **2.** Beagle dogs treated with medroxyprogesterone acetate developed mammary nodules some of which were malignant. Although nodules occasionally appeared in control animals, they were intermittent in nature; whereas the nodules in the drug treated animals were larger, more numerous, persistent, and there were some breast malignancies with metastases. Their significance with respect to humans has not been established. **3.** Discontinue medication pending examination if there is sudden partial or complete loss of vision, onset of proptosis, diplopia, or migraine. If papilledema or retinal vascular lesions occur, withdraw medication. **4.** Detectable amounts of progestin have been identified in the milk of mothers receiving the drug. The effect of this on the nursing infant has not been determined. **5.** Usage in pregnancy is not recommended (see Warning box). **6.** Three major studies in Great Britain and one in this country have shown a statistically significant association between thrombophlebitis, pulmonary embolism, cerebral thrombosis and embolism and the use of oral contraceptives. It has been estimated that users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study indicated that the risk did not persist after discontinuation, and it was not enhanced by long-continued administration.

PRECAUTIONS: A pretreatment physical exam should include special reference to breast and pelvic organs and a Papanicolaou smear. This drug may cause fluid retention; therefore, carefully observe patients with conditions influenced by fluid retention such as epilepsy, migraine, asthma, and cardiac or renal dysfunction. In irregular bleeding per vaginum, bear in mind nonfunctional causes and perform adequate diagnostic measures. Advise pathologist of therapy when submitting relevant specimens. Carefully observe patients with history of psychic depression and discontinue drug if serious depression recurs. Any possible influence of prolonged therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study. Decreased glucose tolerance has been observed in a small percentage of patients on estrogen-progestin combinations; therefore, carefully observe diabetic patients receiving progestin therapy. Age constitutes no absolute limiting factor, although onset of climacteric may be masked. Because of the occasional occurrence of thrombotic disorders (thrombophlebitis, pulmonary embolism, retinal thrombosis, and cerebrovascular disorders) in patients taking estrogen-progestin combinations and since the mechanism is obscure, the physician should be alert to the earliest manifestation of these disorders. (See Patient Information for complete prescribing information.)

ADVERSE REACTIONS: Pregnancy: (see Warning box); **Breast:** rare reports of breast tenderness or galactorrhea; **Skin:** sensitivity reactions including pruritus, urticaria, edema and generalized rash; acne, alopecia and hirsutism in a few patients; **Thromboembolic Phenomena** including thrombophlebitis and pulmonary embolism.

The following adverse reactions have been observed in women taking progestins including medroxyprogesterone acetate: breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; edema; change in weight; changes in cervical erosion and secretions; cholestatic jaundice; rash (allergic) with and without pruritus; mental depression; anaphylaxis and anaphylactoid reactions; pyrexia; insomnia; nausea and somnolence.

A statistically significant association has been demonstrated between use of estrogen-progestin combination drugs and the serious adverse reactions of thrombophlebitis, pulmonary embolism and cerebral thrombosis and embolism. Therefore, patients on progestin therapy should be carefully observed.

Although available evidence is suggestive, a relationship has been neither confirmed nor refuted for the association of the serious adverse reaction of neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been observed in patients receiving estrogen-progestin combination drugs: rise in blood pressure in susceptible individuals; premenstrual-like syndrome; changes in libido; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; fatigue; backache; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; and itching.Therefore, observe patients on progestin therapy carefully.

The following laboratory results may be altered by the use of estrogen-progestin combination drugs: increased sulfobromophthalein retention and other hepatic function tests; coagulation tests (increase in prothrombin factors VII, VIII, IX and X); metyrapone test; pregnanediol determination; thyroid function tests (increase in PBI, and butanol extractable protein bound iodine and decrease in T^3 uptake values).

Caution: Federal law prohibits dispensing without prescription.

MED B-13-S

Upjohn

A Century of Caring
1886·1986

March 1986

J-6144

© 1986 The Upjohn Company

The Upjohn Company, Kalamazoo, MI 49001

A Standard



Provera® 10 MG TABLETS
(medroxyprogesterone acetate)

A Standard in Progesterone Therapy

Please see adjacent page for brief summary of prescribing information.

MOSBY

American Journal of Obstetrics and Gynecology

Founded in 1920

Copyright © 1987 by The C. V. Mosby Company

Contents

August
1987

CLINICAL SECTION

Clinical Opinion

When does a statistical fact become an ethical imperative? 229
Richard K. Silver, M.D., and John Minogue, D.Min.
Chicago, Illinois

Antithyroid drugs: To breast-feed or not to breast-feed 234
David S. Cooper, M.D.
Baltimore, Maryland

Clinical Articles

The use of fetal biophysical profile improves pregnancy outcome in premature rupture of the membranes 236
Anthony M. Vintzileos, M.D., Roy Bors-Koefoed, M.D., James F. Pelegano, M.D., Winston A. Campbell, M.D., John F. Rodis, M.D., David J. Nochimson, M.D., and Vasilios G. Kontopoulos, M.D.
Farmington, Connecticut

(Contents continued on page 7A)

Vol. 157, No. 2, August 1987. The American Journal of Obstetrics and Gynecology (ISSN 0002-9378) is published monthly by The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146. POSTMASTER: Send address changes to The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

1987 Annual subscription rates	U.S.A.	International (surface mail) All regions	International (airmail)* Canada & Mexico	Region 1	Region 2	Region 3
Institutions†	$102.00	$121.00	$142.55	$153.60	$183.85	$214.10
Individuals‡	$ 59.50	$ 78.50	$100.05	$111.10	$141.35	$171.60
Students, residents‡	$ 40.00	$ 59.00	$ 80.55	$ 91.60	$121.85	$152.10

Single copies are $6.50. Remittances should be made by check, draft, or post office or express money order, in U.S. funds drawn through a U.S. bank, payable to this JOURNAL. Claims for missing issues will be serviced only within six months of cover date. Single copy prices will be charged on missing issue claims older than six months from cover date.

*Airmail breakdown—Domestic: First-class and Priority rates for the U.S. and possessions are available upon request. Region 1: Central America, islands, and mainland colonies of European countries in The Americas. Region 2: South America, Europe, Egypt, Africa (bordering the Mediterranean). Region 3: Asia, Australasia, Africa (other than Mediterranean), Middle East, Far East, The Pacific, U.S.S.R. (and constituent Republics).

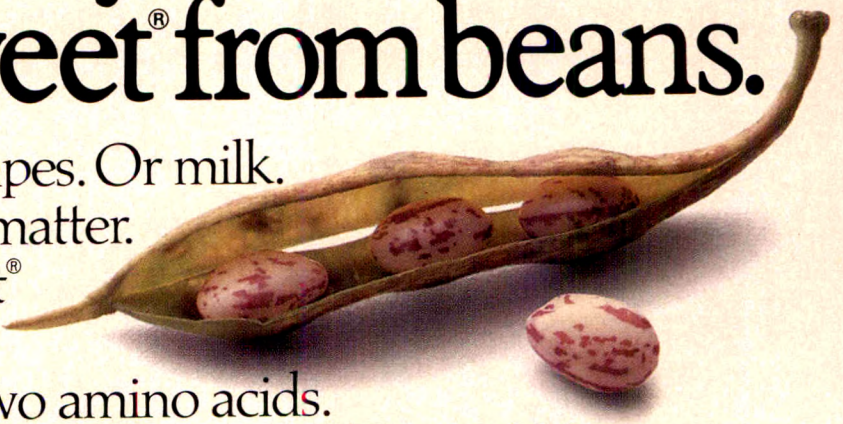
†Institutional (multiple-reader) subscriptions are available to public and private libraries, schools, hospitals, and clinics; city, county, state, provincial, and national government bureaus and departments; and all commercial and private institutions and organizations.

‡Individual subscriptions and all student-rate subscriptions must be in the names of, billed to, and paid by individuals. All student-rate requests must indicate training status and name of institution.

Subscriptions may begin at any time.

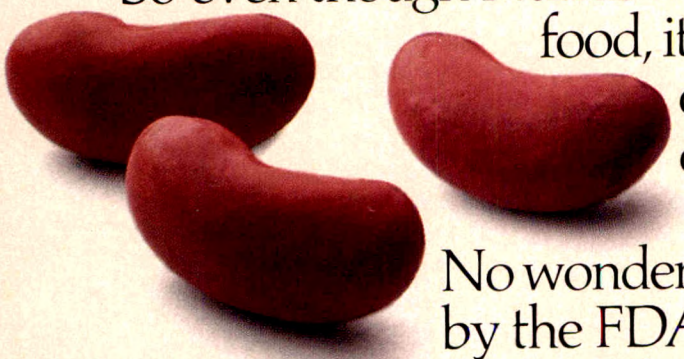
Second-class postage paid at St. Louis, Missouri, and additional mailing offices. Printed in the U.S.A. Copyright © 1987 by The C. V. Mosby Company.

Your body doesn't know NutraSweet® from beans.



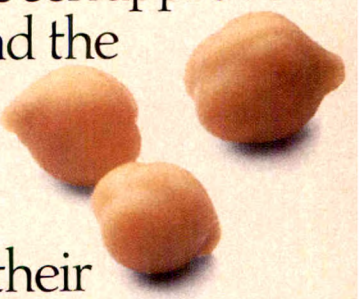
Or peas. Or grapes. Or milk.
Or chicken for that matter.
Because NutraSweet®
brand sweetener is a
dipeptide made of two amino acids.
(Aspartic acid and phenylalanine as the methyl ester.)

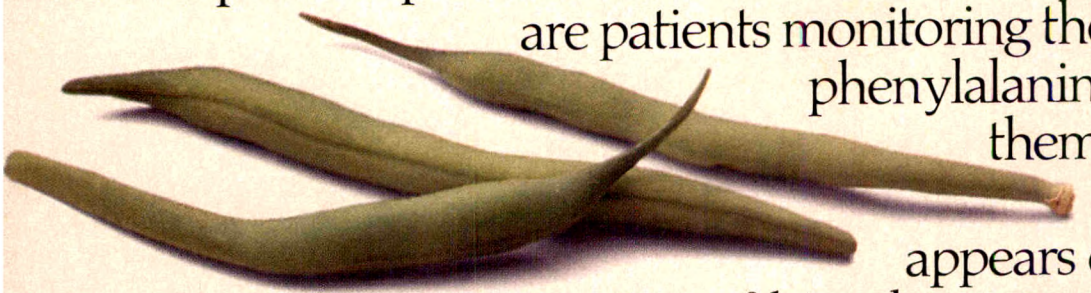
So even though NutraSweet doesn't come from fresh food, it might as well have. Because once past the taste buds, it's digested just like any other simple protein.



No wonder aspartame has been approved by the FDA, the WHO, and the regulatory agencies of more than 50 countries.

The only people who need to limit their consumption of products with NutraSweet, are patients monitoring their phenylalanine intake. For them, an informational label appears on every product containing NutraSweet® brand sweetener.





If you'd like more information, just call 1-800-321-7254.

It's nice to know that with NutraSweet, people who need to cut back on sugar are finding it easier to enjoy something sweet now and then.

NUTRASWEET®
BRAND SWEETENER

Contents continued from page 5A

The effects of maternal ambulation on the nonstress test 240
Lawrence D. Devoe, M.D., Martha Arthur, B.S.N., and Nancy Searle, B.A.
Augusta, Georgia

Fetal monitoring during emergency obstetric transport 245
John P. Elliott, M.D., and Rebecca Trujillo, R.N.
Phoenix, Arizona

Congenital heart block and cardiac anomalies in the absence of maternal connective tissue disease 248
Lewis Shenker, M.D., Kathryn L. Reed, M.D., Caroline F. Anderson, R.D.M.S., Gerald R. Marx, M.D., Richard E. Sobonya, M.D., and Anna R. Graham, M.D.
Tucson, Arizona

Factors affecting psychological adjustment to a fetal death 254
Melody A. Graham, M.A., Suzanne C. Thompson, Ph.D., Mary Estrada, R.N., and Margaret Lynn Yonekura, M.D.
Los Angeles, California

Erb's palsy and fetal bruising after external cephalic version: Case report 258
Boris M. Petrikovsky, M.D., Hema N. DeSilva, M.D., and Fred D. Fumia, M.D.
Farmington and Hartford, Connecticut

A reusable plastic training model for teaching Papanicolaou smear technique 259
Charles R. B. Beckmann, M.D., M.H.P.E., Daniel L. Clarke-Pearson, M.D., and Ray Evenhouse
Chicago, Illinois

Multiple primary gynecologic neoplasms 261
Peter G. Rose, M.D., Edward E. Herterick, B.S., John G. Boutselis, M.D., Melvin Moeshberger, Ph.D., and Larry Sachs, Ph.D.
Columbus, Ohio

The results of cryosurgical treatment of cervical intraepithelial neoplasia at one, five, and ten years 268
J. L. Benedet, M.D., D. M. Miller, M.D., K. G. Nickerson, M.D., and G. H. Anderson, M.B., B.S.
Vancouver, British Columbia, Canada

Host defense during pregnancy: Neutrophil chemotaxis and adherence 274
Peter J. Krause, M.D., Charles J. Ingardia, M.D., Linda T. Pontius, B.S., Harry L. Malech, M.D., Tina M. LoBello, B.A., and Eufronio G. Maderazo, M.D.
Hartford and New Haven, Connecticut

(Contents continued on page 9A)

Satisfaction realized:



87% patient continuation rate† with Ortho ALL-FLEX* Arcing Spring Diaphragm.

Easy for patient to insert—
the Ortho ALL-FLEX* Diaphragm folds easily.

Easy for patient to correctly position in vagina—
the Ortho ALL-FLEX* Diaphragm can be compressed at any two opposing points around the rim.

Easy to use with **GYNOL II* (2% nonoxynol-9) Contraceptive Jelly**—
greaseless, odorless spermicide.

Easy to clean—one-step molding process eliminates gap between spring and dome so jelly or secretions can't accumulate.

Ortho ALL-FLEX*
DIAPHRAGM

†Percentage of patients continuing the method for 12 consecutive months.

Consult the revised patient brochure for new information on Toxic Shock Syndrome (TSS) and wearing time.

ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869-0602

ORTHO

©OPC 1987 OJ-899 *Trademark

Contents continued from page 7A

Varicella zoster antibody testing in the care of pregnant women exposed to varicella 281
James A. McGregor, M.D.C.M., Saralyn Mark, B.A., Gayle P. Crawford, M.D., and Myron J. Levin, M.D.
Denver, Colorado

Sonographic features of the triploid fetus 285
C. Lockwood, M.D., A. Scioscia, M.D., R. Stiller, M.D., and J. Hobbins, M.D.
New Haven, Connecticut

Recurrent small for gestational age birth: Perinatal risks and outcomes 288
Honor M. Wolfe, M.D., Thomas L. Gross, M.D., and Robert J. Sokol, M.D.
Detroit, Michigan, and Cleveland, Ohio

Successful fetal therapy for cystic adenomatoid malformation associated with second-trimester hydrops 294
Steven L. Clark, M.D., Dennis J. Vitale, M.D., Stephen D. Minton, M.D., Ronald A. Stoddard, M.D., and Patty L. Sabey, R.N.
Provo, Utah

Labial agglutination secondary to a herpes simplex II infection 296
Bryan J. DeMarco, M.D., R. Scott Crandall, M.D., and Myroslaw M. Hreshchyshyn, M.D.
Buffalo, New York

Barriers to receiving adequate prenatal care 297
Marilyn L. Poland, Ph.D., R.N., Joel W. Ager, Ph.D., and Jane M. Olson, B.A.
Detroit, Michigan

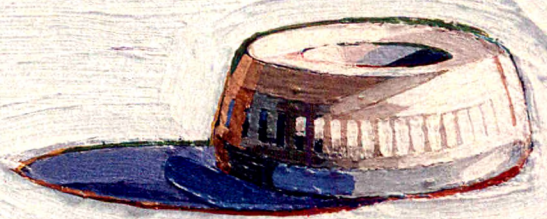
Red blood cell zinc protoporphyrin testing for iron-deficiency anemia in pregnancy 304
Ron B. Schifman, M.D., James E. Thomasson, M.D., and James M. Evers, M.D.
Tucson, Arizona

The normal parturient's admission temperature 308
David B. Acker, M.D., Erica B. Schulman, M.A., Bernard J. Ransil, M.D., Benjamin P. Sachs, M.B., B.S., D.P.H., and Emanuel A. Friedman, M.D., Sc.D.
Boston, Massachusetts

Age-related changes in the female hormonal environment during reproductive life 312
Victoria C. Musey, M.D., Delwood C. Collins, Ph.D., Paul I. Musey, Ph.D., D. Martino-Saltzman, M.S., and John R. K. Preedy, M.D.
Atlanta and Decatur, Georgia

(Contents continued on page 11A)

hCG
RUBELLA
TOXO
STREP A
LH*
CHLAMYDIA*
GONORRHEA*
HERPES*
CANDIDA*
TRICHOMONAS*



YES OR NO. STAT.

This AccuPoint™ Test Disc is virtually a 2-inch lab. As part of the new AccuPoint hCG Pregnancy Test, it offers the ultimate in sensitivity, versatility and convenience by detecting hCG levels as low as 20 mIU/mL—using *either* urine or serum samples.

The same AccuPoint Test Disc is the focus of a growing *system* of non-instrumented, in-office tests offered by Syntex, one of the most trusted names in health care.

All AccuPoint diagnostic tests offer similar protocols, so it's simple to administer a *series* of tests—such as Rubella, Toxo and the hCG Pregnancy Test—for optimum patient care.

Fill out and mail the coupon below for our special offer. And start enhancing your practice now, with the AccuPoint 2-inch lab.

ACCUPOINT™

SYNTEX

YES!

I WANT TO TRY THE ACCUPOINT hCG PREGNANCY TEST KIT FOR HALF PRICE.**

800-523-8990
(In California, 800-428-3003)

☐ Please send me *one* AccuPoint hCG 30-Test Kit and bill me only $59.95 (plus $9.35 handling charge). SIGNATURE

☐ Please contact me with more information on other AccuPoint tests.

Mail to: Syntex Medical Diagnostics, Mail Stop 1-130, 900 Arastradero Road, Palo Alto, CA 94304

NAME

ADDRESS

CITY STATE ZIP

( ) TELEPHONE SPECIALTY

TYPE OF PRACTICE: ☐ GROUP ☐ PARTNERSHIP ☐ SOLO ☐ CLINIC ☐ HOSPITAL

SYNTEX murex™

© 1987, Syntex Medical Diagnostics

Contents continued from page 9A

Evidence that estrogen may be a key factor in hyperprolactinemic anovulation: A case report 318
Hideki Sakamoto, M.D., Konbai Den, M.D., Yasumasa Kondo, M.D., Katsuo Tsubata, M.D., and Shigeo Takagi, M.D.
Tokyo, Japan

Modulating actions of estradiol on gonadotropin-releasing hormone–stimulated prolactin secretion in postmenopausal individuals 320
Elisabeth Christiansen, M.D., Johannes D. Veldhuis, M.D., Alan D. Rogol, M.D., Ph.D., Paul Stumpf, M.D., and William S. Evans, M.D.
Charlottesville, Virginia, and Neptune, New Jersey

Increased thromboxane biosynthesis in normal pregnancy is mainly derived from platelets 325
D. J. Fitzgerald, M.B., G. Mayo, R.N., F. Catella, M.D., S. S. Entman, M.D., and G. A. FitzGerald, M.D.
Nashville, Tennessee

Preeclampsia, delivery, and the hemostatic system 331
Abdelaziz A. Saleh, M.D., Ph.D., Sidney F. Bottoms, M.D., Robert A. Welch, M.D., Abdelkarim M. Ali, M.D., Federico G. Mariona, M.D., and Eberhard F. Mammen, M.D.
Detroit, Michigan

Elevated maternal serum α-fetoprotein levels and oligohydramnios: Poor prognosis for pregnancy outcome 336
Susanna N. Dyer, M.S.N., Barbara K. Burton, M.D., and Lewis H. Nelson, M.D.
Winston-Salem, North Carolina

Mycotic aneurysms in pregnancy 340
Guy M. Boike, M.D., Norman Gove, M.D., Mitchell P. Dombrowski, M.D., Gunter Deppe, M.D., and Robert J. Sokol, M.D.
Detroit, Michigan

A screening test (GBS-Test) for urogenital carriage of group B streptococci 341
Karen Christensen, M.D., Ph.D., and Poul Christensen, M.D., Ph.D.
Lund, Sweden

Fetal growth retardation as a complication of pregnancy in patients with neurofibromatosis 343
Isaac Blickstein, M.D., and Moshe Lancet, M.D.
Rehovot, Israel

γ-Glutamyl transferase and mean cell volume reveal maternal alcohol abuse and fetal alcohol effects 344
Olavi Ylikorkala, M.D., Ph.D., Ulf-Håkan Stenman, M.D., Ph.D., and Erja Halmesmäki, M.D.
Helsinki, Finland

(Contents continued on page 12A)

Contents continued from page 11A

Progesterone synthesis by human amnion, chorion, and decidua at term 349
B. F. Mitchell, M.D., J. R. G. Challis, Ph.D., D.Sc., and L. Lukash, B.Sc.
London, Ontario, Canada

Antepartum predictors of fetal distress in postterm pregnancy 353
Clifford J. Bochner, M.D., Arnold L. Medearis, M.D., Jane Davis, M.D.,
Gary K. Oakes, M.D., Calvin J. Hobel, M.D., and Maclyn E. Wade, M.D.
Los Angeles, California

Ultrasound diagnosis of abruptio placentae with fetomaternal hemorrhage 358
Michael S. Cardwell, M.D.
Rockford, Illinois

Acute atherosis in preeclampsia: Maternal determinants and fetal outcome in the presence of the lesion 360
T. Y. Khong, M.B., M.Sc., J. M. Pearce, and W. B. Robertson, M.D.
London, England

Increased fetoplacental active renin production in pregnancy-induced hypertension 363
Harbinder S. Brar, M.D., Siri L. Kjos, M.D., William Dougherty, M.D.,
Yung-Shun Do, Ph.D., Helen B. Tam, and Willa A. Hsueh, M.D.
Los Angeles, California

Digoxin-like immunoreactive substance in fetuses with and without cardiac pathology 368
C. P. Weiner, M.D., S. Landas, M.D., and T. J. Persoon, M.S.
Iowa City, Iowa

Scalp heat flux and its relationship to scalp blood pH of the fetus 372
Rudolf Rudelstorfer, M.D., Georg Simbruner, M.D., Vinay Sharma, M.B., and
Herbert Janisch, M.D.
Vienna, Austria

Neonatal seizures after cesarean delivery: Higher risk with labor 377
William N. Spellacy, M.D., Paul Q. Peterson, M.D., Ann Winegar, M.S., and
Edward J. Quilligan, M.D.
Chicago, Illinois, and Davis, California

Primary cesarean section and subsquent fertility 379
Anita Parnes LaSala, M.D., and Alan S. Berkeley, M.D.
New York, New York

A study on intrauterine fetal resuscitation with terbutaline 384
Michael S. Patriarco, D.O., Bruce M. Viechnicki, M.D., Thomas A. Hutchinson, M.D.,
Stephen K. Klasko, M.D., and Sze-Ya Yeh, M.D.
Allentown, Pennsylvania

Contents

A randomized trial of ritodrine tocolysis versus expectant management in patients with premature rupture of membranes at 25 to 30 weeks of gestation 388
Thomas J. Garite, M.D., Kirk A. Keegan, M.D., Roger K. Freeman, M.D., and Michael P. Nageotte, M.D.
Long Beach, California

Detection of *Chlamydia trachomatis* cervical infection: A comparison of Papanicolaou and immunofluorescent staining with cell culture 394
Thomas C. Quinn, M.D., Prabodh K. Gupta, M.D., Ronald T. Burkman, M.D., Elizabeth W. Kappus, B.S., Marguerite Barbacci, B.S.N., M.P.H., and Michael R. Spence, M.D., M.P.H.
Bethesda, Maryland

Genetic heterogeneity of prematurity and intrauterine growth retardation: Clues from the Old Order Amish 400
Muin J. Khoury, M.D., Ph.D., and Bernice H. Cohen, Ph.D., M.P.H.
Baltimore, Maryland

Fetal weight estimation formulas with head, abdominal, femur, and thigh circumference measurements 410
Anthony M. Vintzileos, M.D., Winston A. Campbell, M.D., John F. Rodis, M.D., Roy Bors-Koefoed, M.D., and David J. Nochimson, M.D.
Farmington, Connecticut

In utero ponderal index as a prognostic factor in the evaluation of intrauterine growth retardation 415
S. Yagel, D. Zacut, S. Igelstein, Z. Palti, A. Hurwitz, and B. Rosenn
Jerusalem, Israel

Nerve entrapment after Pfannenstiel incision 420
William C. Sippo, M.D., Captain, MC, USA, Anne Burghardt, M.D., Major, MC, USA, and Alphonse C. Gomez, M.D., Colonel, MC, USA
El Paso, Texas

Treatment of polyhydramnios with prostaglandin synthetase inhibitor (indomethacin) 422
Dominique Cabrol, M.D., Robert Landesman, M.D., Joel Muller, M.D., Michele Uzan, M.D., Claude Sureau, M.D., and Brij B. Saxena, Ph.D., D.Sc.
New York, New York, and Paris, France

Human leukocyte antigen associated with endometrial carcinoma with a new classification of endometrial carcinoma based on its etiology 427
Tatsuya Kanuma, M.D., and Masao Igarashi, M.D., Ph.D.
Maebashi, Japan

(Contents continued on page 14A)

Contents continued from page 13A

An evaluation of squamous cell carcinoma antigen in patients with cervical squamous cell carcinoma 433
Elizabeth K. Senekjian, M.D., J. Maitland Young, Ph.D., Patricia A. Weiser, R.N., Cheryl Eck Spencer, M.S., Susan E. Magic, M.A., and Arthur L. Herbst, M.D.
Chicago, Illinois

Neonatal auditory acuity following in utero vibratory acoustic stimulation 440
G. Ohel, M.D., E. Horowitz, M.D., N. Linder, M.D., and H. Sohmer, M.D.
Jerusalem, Israel

Breakage of a fetal scalp blade with retention of fragments in the infant's scalp 441
Phillip Nieburg, M.D., M.P.H., and Steven J. Gross, M.D.
Boston, Massachusetts, and Atlanta, Georgia

Review of causes of perinatal mortality in a regional perinatal center, 1980 to 1984 443
Arne Ohlsson, M.D., Andrew T. Shennan, M.B., and Toby H. Rose, M.D.
Toronto, Ontario, Canada

Mexiletine use in pregnancy and lactation 446
Holly E. Lownes, M.D., and Timothy J. Ives, Pharm.D.
Chapel Hill, North Carolina

BASIC SCIENCE SECTION

Vasopressin-induced bradycardia in fetal and adult sheep is not dependent on an increase in blood pressure 448
Margaret A. Harper, M.D., and James C. Rose, Ph.D.
Winston-Salem, North Carolina

In vivo and in vitro modulation of growth hormone and prolactin of a mixed somatotropic-lactotropic pituitary microadenoma 453
Eberhard K. Muechler, M.D., Ko-En Huang, M.D., Joseph V. McDonald, M.D., and Loretta Tang, Ph.D.
Rochester, New York

Local cerebral glucose utilization in fetal sheep exposed to noise 456
R. M. Abrams, Ph.D., A. A. Hutchison, M.B., Ch.B., K. J. Gerhardt, Ph.D., S. L. Evans, B.S., and J. Pendergast, Ph.D.
Gainesville, Florida

Transplacental stimulation of functional and morphologic fetal rabbit lung maturation: Effect of thyrotropin-releasing hormone 460
Uday Devaskar, M.D., Kenneth Nitta, M.D., Karl Szewczyk, M.D., H. Farouk Sadiq, M.D., and Daphne deMello, M.D.
St. Louis, Missouri

Contents

The effect of severing the spinal cord of fetal lambs on length of gestation 464
Romana A. Nowak, Ph.D., Susan E. Samaras, B.S., Mary C. Chorney, M.S., D.V.M., Daniel R. Hagen, Ph.D., and Philip L. Dziuk, Ph.D.
Urbana, Illinois

The effects of proteolytic enzymes on in vitro ovulation in the rabbit 468
Yasunori Yoshimura, M.D., Rosemary Santulli, B.S., Susan J. Atlas, Ph.D., Shingo Fujii, M.D., and Edward E. Wallach, M.D.
Baltimore, Maryland

Current Development

Disruption of embryonic and fetal development due to preimplantation chemical insults: A critical review 476
Philip M. Iannaccone, M.D., Ph.D., Nancy L. Bossert, B.A., and Camille S. Connelly, B.A.
Chicago, Illinois

SYMPOSIUM ON INFECTION: A COMPLICATION OF OBSTETRIC AND GYNECOLOGIC SURGERY

Treatment and prevention of female pelvic infection: The quest for single-agent therapy 485
F. Gary Cunningham, M.D.
Dallas, Texas

Single-agent therapy for women with acute polymicrobial pelvic infections 488
David L. Hemsell, M.D., Molly C. Heard, R.A., Brenda J Nobles, and Patricia G. Hemsell, R.A.
Dallas, Texas

Changing concepts in obstetric antibiotic prophylaxis 491
Rudolph P. Galask, M.D.
Iowa City, Iowa

Single-dose prophylaxis for vaginal and abdominal hysterectomy 498
David L. Hemsell, M.D., Molly C. Heard, R.A., Brenda J. Nobles, Roger E. Bawdon, Ph.D., and Patricia G. Hemsell, R.A.
Dallas, Texas

Single-dose versus multiple-dose piperacillin prophylaxis in primary cesarean operation 502
Stanley A. Gall, M.D., and Gale B. Hill, Ph.D.
Chicago, Illinois, and Durham, North Carolina

(Contents continued on page 16A)

Contents continued from page 15A

Cost-effective use of antibiotic prophylaxis for cesarean section 506
Larry C. Ford, M.D., Hunter A. Hammil, M.D., and Thomas B. Lebherz, M.D.
Los Angeles, California

Questions from the audience 511

CORRESPONDENCE

Profound hypotension complicating magnesium therapy Emeric P. Frohlich, Dr.Med. *Johannesburg, South Africa* 513

Fetal heart rate monitoring G. S. Dawes and C. W. G. Redman *Headington, Oxford, England* 513

Reply Teiichiro Fukushima, M.D., Ezra C. Davidson, Jr., M.D., and Edward H. Hon, M.D. *Los Angeles, California* 514

Recurrent spontaneous abortion and human leukocyte antigen DRw8 Masaru Takamizawa, M.D., Takeo Juji, M.D., Hiroko Tsuneyoshi, Ph.D., Mie Nieda, Ph.D., Tomoyuki Fujii, M.D., Takashi Kawana, M.D., and Masahiko Mizuno, M.D. *Tokyo, Japan* 514

Myocardial infarction in the puerperium Harold N. Feldman, M.D. *East Hanover, New Jersey* 515

Reply Leslie Iffy, M.D., Willem ten Hove, M.D., and Garry Frisoli, M.D. *Newark, New Jersey* 516

Venous thrombosis in patients with cardiac pacemakers and oral contraceptive use Gopal Rao, M.D. *Pittsburgh, Pennsylvania* 516

Femininity—a problem(?) Eduard Eichner, M.D. *Shaker Heights, Ohio* 517

Definitions of sensitivity, specificity, false negative results, and false positive results Mark D. Kolins, M.D. *Detroit, Michigan* 517

***Haemophilus influenzae* infection and midtrimester abortion** Marvin S. Amstey, M.D., and Edward Ogden, M.D. *Rochester, New York* 518

BOOKS

Books received 519

ITEMS

Items 520

Information for authors on pages 23A, 24A, and 25A

Index to advertisers on page 39A

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by The C. V. Mosby Company for libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that 10¢ per page is paid directly to CCC, 29 Congress St., Salem, MA 01970; phone (617)744-3350. The U.S. Government retains a nonexclusive, royalty-free license in and to any copyright on articles authored by government employees. 0002-9378/87 $00.00 + .10.

HOW MANY NEW DRUG ADDICTS HAVE YOU DELIVERED?



Even one more addict adds to the staggering number of drug abusers in our society. You, as a physician, are in a unique position to help reduce this number. Just ask your patient a simple question during her initial workup: *Are you using drugs?* Asking about drug use is no more an invasion of privacy than asking about exposure to German measles. It's just as important to her baby's health. And knowing about it may prevent serious problems for you, mother, and baby. Help free this generation—and the next—from drugs.
Remember...

You'll never know unless you ask.

PARTNERSHIP FOR A DRUG FREE AMERICA

American Journal of Obstetrics and Gynecology

Editors

Editors

JOHN I. BREWER, *Editor in Chief*
Sandra Fernandez, *Senior Editorial Assistant*

FREDERICK P. ZUSPAN, *Editor*
Meta M. Peterson, *Editorial Assistant*

E. J. QUILLIGAN, *Editor*
Rosalyn Vu, *Editorial Assistant*

ALBERT B. GERBIE, *Associate Editor*

Advisory committee on policy

William R. Anderson
Mason C. Andrews
E. Forrest Boyd
Douglas W. Cudmore
A. Jerome Freeland
Steven G. Gabbe
Edgar O. Horger III
W. Glenn Hurt
Walter S. Keifer
Charles F. Langmade
Roger A. Lobo
Douglas J. Marchant
James H. Maxwell
Arthur M. McCausland
Paul G. McDonough
James A. Merrill
Frank C. Miller
Daniel R. Mishell
Patrick T. Mohide
John C. Morrison
John Read
Keith P. Russell
Ruth W. Schwartz
Stacy R. Stephens
Ruth Stockdale
Henry A. Thiede
Edward E. Wallach
Anne C. Wentz

Board of corresponding editors

Stuart Campbell, *England*
Luigi Carenza, *Italy*
Eliahu Sadovsky, *Israel*
Nils-Otto Sjöberg, *Sweden*
Masakuni Suzuki, *Japan*

Publisher
THE C. V. MOSBY COMPANY, 11830 Westline Industrial Drive, St. Louis, Missouri 63146
Kay G. Goehler, *Senior Manuscript Editor*

Medical Staff Fellowships In Genetics, Endocrinology, And Developmental Biology At NIH

The National Institute of Child Health and Human Development of the National Institutes of Health is offering a limited number of three-year Medical Staff Fellowship positions to candidates of the highest caliber. Fellowships begin July 1, 1989 and 1990. Positions are available for physicians who have completed at least two years of training in Internal Medicine, Pediatrics, or Obstetrics and Gynecology. Other medical specialty credentials will be considered on a case by case basis. Candidates must be United States citizens or permanent residents; graduates of foreign medical schools must be certified by the ECFMG. (Non-citizens wishing only laboratory experience may apply for the Fogarty Visiting Fellowship and do not need ECFMG certification.)

The training program consists either of one clinical year and two years of laboratory-based research (satisfying the requirements for the Subspecialty Boards in Human Genetics, Adult or Pediatric Endocrinology, or Gynecologic Endocrinology), or three years of laboratory research exclusively. The endocrinology fellowships are integrated with the NIH Interinstitute Endocrinology Fellowship Program, and the human genetics fellowships are integrated with the NIH Interinstitute Medical Genetics Program. The gynecologic-endocrinology fellowship is integrated with programs at the Walter Reed Hospital and the National Naval Medical Center.

For those selecting clinically oriented fellowships, research experience will be obtained in the Laboratory or Branch of the Fellow's choice from among the Institute's three clinical research Branches and nine basic science Laboratories. For those candidates selecting laboratory research exclusively, these same choices are available.

The major emphasis of the NICHD Intramural Research Program is on basic and clinically applied research in the broad area of developmental biology. Areas presently being examined include developmental endocrinology, reproductive biology, cellular differentiation, human genetics and inborn metabolic errors, molecular endocrinology, developmental and molecular immunity, developmental neurobiology, molecular genetics (bacteria, virus, yeast, drosophila, and animal cell), pharmacogenetics, growth factors, neurochemistry and neuroimmunology, theoretical and physical biology, cell biology (especially receptors and organelles), and comparative ethology.

For physicians interested in pursuing a career in academic medicine and research, these Fellowships offer an opportunity to train with scientists and physicians of international stature. Excellent facilities are available on the 306-acre NIH campus in Bethesda, Maryland, including a 480-bed research and teaching hospital, more than 1,000 laboratories, and the National Library of Medicine. Medical Staff Fellows receive an initial salary of $30,000 with increments of $2,000 per year. Moving and traveling allowances, and Federal health care benefits are available.

Interested candidates should send their curriculum vitae to (or request further information from):

Arthur S. Levine, M.D., Scientific Director,
National Institute of Child Health and Human Development,
National Institutes of Health, 9000 Rockville Pike,
Building 31, Room 2A50, Bethesda, Maryland 29892, (301) 496-2133

NIH is an Equal Opportunity Employer.

Deadline for applications is November 15, 1987.

NATIONAL INSTITUTES OF HEALTH

CHARLES C THOMAS • PUBLISHER

COLPOSCOPY: A Scientific and Practical Approach to the Cervix, Vagina and Vulva in Health and Disease (3rd Ed.) *by* **Malcolm Coppleson, Ellis Pixley** *and* **Bevan Reid.** Precise histological correlations, excellent atlas features, and a sound approach to detection and management make this book essential reading to all who practice colposcopy. *'86, $71.25*

CLEFT LIP AND PALATE: Aspects of Reproductive Biology *by* **Krishna R. Dronamraju.** In this summary of his own and others' research into the etiology of oral-facial clefts, the author emphasizes prenatal selection in relation to a predisposition to malformation. He also discusses fetal loss in families evidencing clefts and explores practical applications. *'86, $27.25*

VAGINAL HYSTERECTOMY: Indications, Technique, and Complications (3rd Ed.) *by* **Laman A. Gray.** This thorough Third Edition encompasses indications for vaginal hysterectomy; discusses the use of adrenalin to conserve blood; recommends prophylactic antibiotics; and details complications related to cystocele and rectocele repair. *'83, $53.75*

We fill all orders promptly • Books sent on approval
MasterCard, Visa & prepaid orders sent postpaid
Catalog sent on request • Write or call (217) 789-8980

2600 South First Street
Springfield • Illinois • 62794-9265

Ob/Gyn Videotapes
$95.00 each

Informed Consent Systems
Videotapes:

✓reflect current patient education trends
✓offer improved legal protection for the doctor
✓use easy to understand diagrams and graphics
✓save time for the doctor and staff

Buy 8 videotapes and get a Free Videotape Player

Preview Videotapes are $15.00 each

Interested?

Call 1-800-FILMTEC

Informed Consent Systems, Distributed by
FILMTEC, Inc., Reston, VA 22090

Societies

in addition to those listed on the front cover
the Journal is the official publication
of the following societies

NEW YORK OBSTETRICAL SOCIETY

OBSTETRICAL SOCIETY OF PHILADELPHIA

BROOKLYN GYNECOLOGICAL SOCIETY

ST. LOUIS GYNECOLOGICAL SOCIETY

NEW ORLEANS GYNECOLOGICAL AND OBSTETRICAL SOCIETY

THE OBSTETRICAL AND GYNECOLOGICAL SOCIETY OF MARYLAND

CHICAGO GYNECOLOGICAL SOCIETY

CINCINNATI OBSTETRICAL AND GYNECOLOGICAL SOCIETY

WASHINGTON GYNECOLOGICAL SOCIETY

PITTSBURGH OBSTETRICAL AND GYNECOLOGICAL SOCIETY

BOSTON OBSTETRICAL SOCIETY

LOUISVILLE OBSTETRICAL AND GYNECOLOGICAL SOCIETY

SEATTLE GYNECOLOGICAL SOCIETY

ALABAMA ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

AKRON OBSTETRICAL AND GYNECOLOGICAL SOCIETY

KANSAS CITY GYNECOLOGICAL SOCIETY

CENTRAL NEW YORK ASSOCIATION OF GYNECOLOGISTS
AND OBSTETRICIANS

NEW JERSEY OBSTETRICAL AND GYNECOLOGICAL SOCIETY

IOWA OBSTETRIC AND GYNECOLOGIC SOCIETY

THE TEXAS ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

OKLAHOMA CITY OBSTETRICAL AND GYNECOLOGICAL SOCIETY

MEMPHIS OBSTETRICAL AND GYNECOLOGICAL SOCIETY

UTAH OBSTETRICAL AND GYNECOLOGICAL SOCIETY

ROCHESTER OBSTETRICAL AND GYNECOLOGICAL SOCIETY

ARKANSAS OBSTETRICAL AND GYNECOLOGICAL SOCIETY

TENNESSEE STATE OBSTETRICAL AND GYNECOLOGICAL SOCIETY

NEW YORK GYNECOLOGICAL SOCIETY

PACIFIC NORTHWEST OBSTETRICAL AND GYNECOLOGICAL ASSOCIATION

BUFFALO OBSTETRICAL AND GYNECOLOGICAL SOCIETY

SAN FRANCISCO GYNECOLOGICAL SOCIETY

JACKSON GYNECIC SOCIETY

INDIANA OBSTETRICAL AND GYNECOLOGICAL SOCIETY

THE MINNESOTA OBSTETRICAL AND GYNECOLOGICAL SOCIETY

Faculty Position in Maternal-Fetal Medicine

The Department of Obstetrics and Gynecology at Wright State University School of Medicine invites applications for a full-time faculty position in Maternal-Fetal Medicine combined with the position of Medical Director of a regional perinatal care center.

Primary duties will include the teaching of medical students and residents, the pursuit of scholarly activities, and the medical administration of the perinatal center which includes the obstetrical unit and the labor and delivery suite.

Applicant qualifications include the M.D. degree, board certification in obstetrics and gynecology, licensibility to practice Medicine in Ohio, completion of a fellowship in maternal-fetal medicine, board eligibility or certification in maternal-fetal medicine, and extensive training and experience in perinatal medicine and administration of health services. Academic rank and salary will be commensurate with qualifications and professional experience. Please send curriculum vitae and the names of three references to: John J. Halki, M.D., Ph.D., Chair and Program and Director, Department of Obstetrics and Gynecology, Wright State University School of Medicine, 1 Wyoming Street, Dayton, OH 45409.

The Wright State University School of Medicine is an Affirmative Action and Equal Opportunity Employer.

All applications received by September 30, 1987 will be considered; if the position is not filled therefrom; applications will be considered as received until the position is filled.

OB-GYN

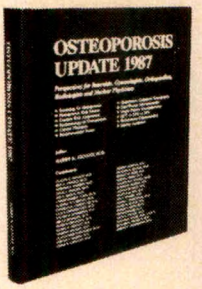
OB-GYN TEACHING FACULTY AT TRUMAN Medical Center/East, one of two teaching hospitals for the University of Missouri-Kansas City School of Medicine. Joint faculty appointments in Departments of Family Medicine and OB-GYN. Primary responsibilities teaching OB and office GYN to Family Practice residents, performing GYN surgery, some lesser involvement with medical students, and consultation in high-risk pregnancy problems as they present. Competitive salary, excellent fringes, opportunities for additional practice, if desired. Contact Family Medicine Department, University of Missouri-Kansas City School of Medicine, Truman Medical Center-East, 7900 Lee's Summit Road, Kansas City, MO 64139. (816) 373-6228. Attention: Thomas A. Nicholas, M.D., Chairman.

NEW PUBLICATION

OSTEOPOROSIS UPDATE 1987

Perspectives for Internists, Gynecologists, Orthopaedists, Radiologists and Nuclear Physicians

- **Screening for Osteoporosis**
- **Osteoporosis Risk Factors**
- **Fracture Risk Assessment**
- **Epidemiology of Osteoporosis**
- **Current Therapies**
- **Reimbursement Issues**
- **Quantitative Computed Tomography**
- **Dual Photon Absorptiometry**
- **Single Photon Absorptiometry**
- **QCT vs DPA vs SPA**
- **Performance Characteristics**
- **Quality Assurance**



Editor: **HARRY K. GENANT, M.D.**
Professor of Radiology, Medicine and Orthopaedic Surgery
Chief of Skeletal Section
University of California School of Medicine
San Francisco, California

Contributors: **Claude D. Arnaud, M.D.; Ben A. Arnold, Ph.D.; Howard S. Barden, Ph.D.; David J. Baylind, M.D.; Jon E. Block, Ph.D.; Christopher E. Cann, Ph.D.; Russell W. Chesney, M.D.; Charles H. Chestnut III, M.D.; Claus Christiansen, M.D.; Steven R. Cummings, M.D.; Bruce Ettinger, M.D.; Hossein Firozznia, M.D.; Harry R. Genant, M.D.; Vincent Gilsanz, M.D.; Claus-Christian Gluer, Ph.D.; Steven T. Harris, M.D.; Cesar R. Libanati, M.D.; Robert Marcus, M.D.; Richard B. Mazess, Ph.D.; Robert M. Neer, M.D.; Russell Nord, Ph.D.; Malcolm R. Powell, Ph.D.; Robert R. Recker, M.D.; Donald Resnick, M.D.; Bente Juel Riis, M.D.; Lee F. Rogers, M.D.; Daniel I. Rosenthal, M.D.; David M. Slovik, M.D.; Rebecca Smith, B.S.E.; Peter Steiger, Ph.D.; Arch K. Taylor, Ph.D.; John Max Vogel, M.D., F.A.C.S.; Richard D. Wasnich, M.D.; Robert G. Zamenhoff, Ph.D.**

ORDER FORM *(Prepayment Required)*

☐ Please send me _____ copies of **Osteoporosis Update 1987** @ $79.00 per copy.
Residents of California — Please add $5.14 tax
CHECK MUST BE IN U.S. DOLLARS
Postage and Handling Included

☐ A. Check — Payable to **Radiology Research and Education Foundation (RREF)**

☐ B. VISA #_____/_____/_____/_____
Expiration Date _____

☐ C. MasterCard #_____/_____/_____/_____
Expiration Date _____

Name _____

Address _____

City _____ State _____

Zip _____ Daytime Phone (_____) _____

Signature _____

Mail to: **Radiology Postgraduate Education**
Registration Office
University of California, Room 569-U
San Francisco, California 94143-0958
(415) 476-5808

MOSBY Medical Journals

Medical specialty publications bringing important medical literature to over 205,000 worldwide subscribers throughout the year. MOSBY journals provide information progressive physicians need to maintain a continuing education program in their specialty areas. **SAMPLE COPIES of journals are available.**

* **THE JOURNAL *of* ALLERGY *and* CLINICAL IMMUNOLOGY**
Monthly. Institutions: $92.00; Individuals: $45.00; Students, residents: $32.00. Official publication of the American Academy of Allergy and Immunology.

CLINICAL NUTRITION
Bimonthly. Institutions: $39.00; Individuals: $39.00; Students, residents: $21.00.

* **CLINICAL PHARMACOLOGY *and* THERAPEUTICS**
Monthly. Institutions: $113.00; Individuals: $65.00; Students, residents: $45.00. Official publication of the American Society for Pharmacology and Experimental Therapeutics and the American Society of Clinical Pharmacology and Therapeutics.

* **JOURNAL *of* THE AMERICAN ACADEMY *of* DERMATOLOGY**
Monthly. Institutions: $104.00; Individuals: $57.00; Students, residents: $36.00. Official publication of the American Academy of Dermatology.

* **THE JOURNAL *of* HAND SURGERY (American Volume)**
Bimonthly. Institutions: $85.00; Individuals: $38.50; Students, residents: $25.00. Official publication of the American Society for Surgery of the Hand.

* **AMERICAN HEART JOURNAL**
Monthly. Institutions: $98.00; Individuals: $51.00; Students, residents: $33.00.

* **HEART & LUNG: THE JOURNAL *of* CRITICAL CARE**
Bimonthly. Institutions: $68.00; Individuals: $21.00; Students, residents: $16.00.

* **AMERICAN JOURNAL *of* INFECTION CONTROL**
Bimonthly. Institutions: $66.00; Individuals: $19.75; Students, residents: $15.00. Official publication of the Association for Practitioners in Infection Control.

* **THE JOURNAL *of* LABORATORY *and* CLINICAL MEDICINE**
Monthly. Institutions: $109.00; Individuals: $62.00; Students, residents: $35.00. Official publication of the Central Society for Clinical Research.

* **AMERICAN JOURNAL *of* OBSTETRICS *and* GYNECOLOGY**
Monthly. Institutions: $102.00; Individuals: $59.50; Students, residents: $40.00. Official publication of the American Board of Obstetrics and Gynecology, The American Gynecological and Obstetrical Society, plus 32 regional societies.

* **OTOLARYNGOLOGY—HEAD *and* NECK SURGERY**
Monthly. Institutions: $115.00; Individuals: $70.00; Students, residents (non-Academy members): $45.00; Residents (Academy members): $20.00. Official publication of the American Academy of Otolaryngology–Head and Neck Surgery, the American Academy of Otolaryngic Allergy, and the American Neurotology Society.

* **THE JOURNAL *of* PEDIATRICS**
Monthly. Institutions: $95.00; Individuals: $47.50; Students, residents: $30.00.

* **SURGERY**
Monthly. Institutions: $97.00; Individuals: $49.50; Students, residents: $32.00. Official publication of the Society of University Surgeons, Central Surgical Association, and the American Association of Endocrine Surgeons.

* **THE JOURNAL *of* THORACIC *and* CARDIOVASCULAR SURGERY**
Monthly. Institutions: $118.00; Individuals: $70.00; Students, residents: $45.00. Official publication of the American Association for Thoracic Surgery and the Western Thoracic Surgical Association.

* **JOURNAL *of* VASCULAR SURGERY**
Monthly. Institutions: $98.00; Individuals: $57.00; Students, residents: $35.00. Official publication of the Society for Vascular Surgery and the International Society for Cardiovascular Surgery (North American Chapter).

THE JOURNAL *of* HEART TRANSPLANTATION *new*
Bimonthly. Institutions: $60.00; Individuals: $40.00; Students, residents: $25.00. Official publication of the International Society for Heart Transplantation.

* Included in Index Medicus

AJS-078

A Tradition of Publishing Excellence

Subscription rates quoted are for USA only. International rates (surface and airmail delivery) available upon request. All subscriptions are for one-year terms. Subscriptions may begin at any time.

THE C.V. MOSBY COMPANY
Journal Circulation Department
11830 Westline Industrial Drive
St. Louis, Missouri 63146

In the USA, call toll-free:
1-800-325-4177, ext. 351
In Missouri, call collect:
(314) 872-8370, ext. 351

American Journal of Obstetrics and Gynecology

Copyright © 1987 by The C. V. Mosby Company

Information for Authors

Editors

John I. Brewer, *Editor in Chief*
710 North Fairbanks Court, Suite 8300,
Chicago, IL 60611

Sandra Fernandez, *Senior Editorial Assistant*
Tel.: (312) 908-5266

Frederick P. Zuspan, *Editor*
The Ohio State University, Fifth Floor, Means Hall,
1654 Upham Dr., Columbus, OH 43210

Meta M. Peterson, *Editorial Assistant*
Tel.: (614) 293-8798

E. J. Quilligan, *Editor*
University of California, Irvine, California College of Medicine, Med Surge 1, Room 118, Irvine, CA 92717

Rosalyn Vu, *Editorial Assistant*
Tel.: (714) 856-5092

Albert B. Gerbie, *Associate Editor*
710 North Fairbanks Court, Suite 8300,
Chicago, IL 60611

Publisher
THE C. V. MOSBY COMPANY, 11830 Westline Industrial Drive, St. Louis, Missouri 63146

Kay G. Goehler, *Senior Manuscript Editor*
Tel.: (314) 872-8370

INFORMATION FOR AUTHORS

Editorial policies

The requirements for manuscripts submitted to the *American Journal of Obstetrics and Gynecology* conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors and published in *Annals of Internal Medicine* 1982;96(6):766-71. Certain requirements unique to our JOURNAL are provided in Information for Authors, published in each issue of the JOURNAL, and in more detail in the *Guide to Writing for the American Journal of Obstetrics and Gynecology*. The latter may be obtained from The C. V. Mosby Company or the Editors on request.

Manuscripts should be submitted to one of the three Editors as follows: (1) Dr. Brewer—all articles originating from the southeastern quadrant of the United States or Canada and those presented before one of the official sponsoring societies, except the Society for Gynecologic Investigation and The Society of Perinatal Obstetricians; (2) Dr. Zuspan—articles from the northeastern quadrant of the United States, Japan, Israel, Italy, and England, articles written for Clinical Opinion and Current Development, and Letters to the Editors; (3) Dr. Quilligan—articles from the north central states (including Ohio), states west of the Mississippi, Hawaii, Alaska, and abroad (except Japan, Israel, Italy, and England). Dr. Zuspan is responsible for articles from the Society for Gynecologic Investigation. Manuscripts should be submitted to Dr. Roger A. Lobo, Women's Hospital, 1240 North Mission Road, Room 1M2, Los Angeles, CA 90033. Dr. Quilligan is responsible for articles from The Society of Perinatal Obstetricians. Manuscripts should be submitted to Dr. John A. Read, 9102 Lake Steilacoom Point Road, S.W., Tacoma, WA 98498.

Most of the provisions of the Copyright Act of 1976 became effective on January 1, 1978. **Therefore, all manuscripts must be accompanied by the following written statement, signed by one author:** "The undersigned author transfers all copyright ownership of the manuscript [title of article] to The C. V. Mosby Company in the event the work is published. The undersigned author warrants that the article is original, is not under consideration by another journal, and has not been previously published. I sign for and accept responsibility for releasing this material on behalf of any and all co-authors." Authors will be consulted, when possible, regarding republication of their material.

When submitting a manuscript, an author should include, if applicable, a full statement about all submissions and prior reports in any medium that might be regarded as prior or duplicate publication of the same or similar work. Copies of such material should be included with the submitted manuscript. Articles previously published in another language will not be considered.

It is assumed by the Editors that articles emanating from a particular institution are submitted with the approval of the requisite authority. Articles dealing with human experimentation that require local institutional approval must have this approval prior to submission of the article for publication and it should be so indicated in the Methods section. Reports of experiments on animals must indicate which guidelines for the care and use of the animals were followed.

Statements and opinions expressed in articles and communications herein are those of the author(s) and not necessarily those of the Editor(s) or Publisher and the Edi-

tor(s) and Publisher disclaim any responsibility or liability for such material. Neither the Editor(s) nor the Publisher guarantees, warrants, or endorses any product or service advertised in this publication, nor do they guarantee any claim made by the manufacturer of such product or service.

General requirements for preparation of manuscripts

The original and two good-quality copies of the manuscript and three glossy prints of illustrations are required.

Manuscripts must be typed double spaced on one side only on 22 by 28 cm (8 1/2 by 11 inch) white bond paper with 1 inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, abstract, body of text, references, legends, and tables.

Title page. The title page should contain in sequence the title (concise and suitable for indexing purposes); author line with first name, middle initial, and last name of all authors and their highest academic degree (both M.D. and Ph.D. are acceptable); city(ies), state(s) in which the study was conducted; divisional, or departmental, and institutional affiliations at the time the study was performed; acknowledgement of source(s) of support; presented line, if applicable; disclaimers, if any; name and address of author to whom requests for reprints should be addressed (if reprints will not be available, it should be so stated); and name and address of author responsible for correspondence concerning the manuscript if different from author to whom reprint requests are addressed. At the bottom of the title page supply a short title for the running head not exceeding 52 characters (including word spaces).

Abstract page and key words/phrases. On manuscript page two is the abstract, also typed double spaced with the required margins and headed by the title of the article and author(s) name(s). Abstracts for regular articles, Current Investigation, Clinical Opinion, and Current Development may not exceed 150 words. Abstracts for case reports and brief communications may not exceed 50 words. Below the abstract list 3 to 5 *key words* or short phrases for indexing purposes.

Text. Do not hesitate to write your manuscript in the first-person, active voice if it is more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations since the emphasis is on the "action" rather than the person performing the action.

Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors*. Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify Material and Methods, Results, and Comment. Authors may wish to summarize their findings in a short paragraph at the end of the Comment section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods section* describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results section* present the detailed findings. Include mentions of all tables and/or figures. Avoid duplication of text and supporting material. Emphasize only your important observations; do not compare your observations to those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment section* state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgments. Acknowledge only persons who have made substantive contributions to the study.

References. A reasonable number, except for case reports and brief communications, limited to 2, and Current Development, for which there is no limit. Number references consecutively in the order in which they are mentioned in the text. Use the format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals." Journal titles should conform to abbreviations used in *Cumulated Index Medicus*.

Illustrations and tables. A reasonable number of halftone and line illustrations will be reproduced without charge, but special arrangements must be made with the Editors for color reproduction. For color photographs, submit original transparencies and two sets of unmounted prints on glossy paper with good color balance. For black and white illustrations, submit three sets of 3 by 4 inch (minimum) to 5 by 7 inch (maximum) unmounted glossy photographic prints. Figures must be cited consecutively in the text in Arabic numerals and identified thusly on the back of the print (gummed label with author(s) name(s), title of article, number, and top marked clearly). Do not use paper clips. Legends must accompany all figures; type double spaced on a single sheet of paper if possible and number with manuscript in sequence after the references. Titles should be included in the legend, *not* on the print.

All lettering must be done with commercially available paste-on letters (or numbers) or by a professional; typed or freehand lettering is not acceptable. All lettering must be in proportion to the drawing, graph, or photograph. Original drawings, appropriately done in black India ink, roentgenograms, and other material must be submitted as glossy photographic prints with good black and white contrast. Consistency in size within the article is strongly preferred. Any special instructions regarding sizing should be clearly noted. Illustrations will be returned only on the author's request.

Tables should be typed on separate sheets of paper and

Estimating length of manuscript

The length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A 4200 word text can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7 page article in the JOURNAL. The 2000 words of text alone make approximately 8 pages of manuscript typed double spaced with the required 1 inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 typed words in manuscript. Thus if a greater number of illustrations and tables are used, the length of the text should be adjusted accordingly.

included at the end of the text, one to a page. They should be numbered in Roman numerals. Each table must be cited in sequence at an appropriate point in the text. Captions should be brief yet indicate clearly the purpose or content of each table and each column precisely defined by headings. Abbreviations and special designations should be explained in a footnote to the table. Special arrangements must be made with the Editors for elaborate tables because of space limitations.

Illustrations and tables should supplement, not duplicate, the text; presentation in either one or the other will suffice.

Permissions and photographic consent. Previously published tables and figures must be correctly referenced and written permission from the copyright holder included with the manuscript. Photographs of identifiable persons must be accompanied by signed releases or else all recognizable features masked.

Requirements for special sections

Case reports and brief clinical and basic science communications. Limit of 700 words, 2 references. Include abstract of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures are used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

Current Investigation. Same requirements as for a regular article.

Clinical Opinion. Limit of 3000 words, 16 references. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Current Development. Limit of 6000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Correspondence. Two types of correspondence will be considered for publication. (1) A Letter to the Editors commenting on an article that has appeared in the JOURNAL should be brief and directly related to the published article. The editorial staff reserves the right to shorten letters if necessary and to make minor editorial alterations without reference to the writer. Letters may be published together with a reply from the original author. As space for letters is limited, only a selection of letters submitted may be published. (2) A brief case presentation or a short report of a pertinent observation in the form of a Letter to the Editors will be considered for publication. All letters should be typed double spaced. Letters should be sent to Dr. Zuspan.

Announcements. Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

Books. Books received will be listed in the JOURNAL. They should be sent to Dr. Gerbie. No books will be returned.

Reprints. Reprints of articles must be obtained from the author. The corresponding author will receive a price schedule and order form at the time of publication. Reprints in quantities must be ordered from the Publisher with the author's consent.

Business communications. Communications of a business nature and all advertising communications should be addressed to: Journal Publisher, The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, or call Journal Advertising Production Manager (314) 872-8370.

Checklist

—Letter of submission
—Copyright transfer letter
—Original and two xerographic copies of manuscript
—Title page
—Title of article
—Full name(s) and affiliations of author(s)
—Author to whom correspondence is to be sent
—Reprint request line or line stating reprints not available
—Short title
—Abstract (double spaced), 3 to 5 key words/phrases
—Article proper (double spaced)
—References (double spaced), on a separate sheet
—Legends (double spaced), on a separate sheet
—Tables (double spaced), each on a separate sheet
—Illustrations, properly labeled (three copies of glossy prints)
—Permission to reproduce published material

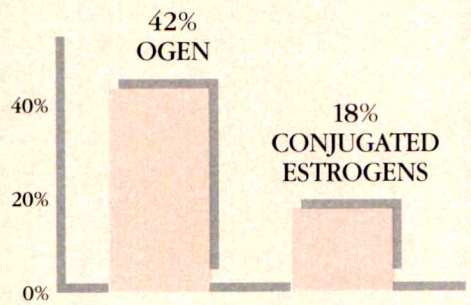
Withdrawal Bleeding in the ERT Patient:

Necessity or Nuisance?

- **To protect against the endometrial hyperplasia** that may result from continuous, unopposed estrogen administration, cyclical estrogen/progestogen therapy is often given.[1] But this may induce withdrawal bleeding, unacceptable to many patients.

- **Progestogen initiates bleeding** only when the endometrial mucosa is stimulated to the threshold level by the estrogen.

More women on Ogen free of withdrawal bleeding[2]



Patients received Ogen 1.25 (n=45) or conjugated equine estrogens 0.625 mg (n=50) days 1-24 over 3 monthly cycles. All patients also received a progestational agent days 15-24. Adapted from Wren, et al.[2]

- Studies[2,3] suggest that Ogen® (estropipate) produces less withdrawal bleeding...*at doses that effectively protect patients from symptoms of menopause.*

7013807R

Abbott Pharmaceuticals, Inc.
North Chicago, Illinois 60064



OGEN® (estropipate) Induces Less Withdrawal Bleeding

OGEN®
(estropipate)

Only estrone. Identical to the body's own.

OGEN®

ESTROPIPATE TABLETS, USP

Tablets

WARNING:

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. OGEN SHOULD NOT BE USED DURING PREGNANCY.

According to some investigators, the use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. Studies have reported that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. In one of these studies, this risk was estimated as not greater than 4 per 1000 exposures. Furthermore, there are reports that a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these reported changes are histologically benign, the investigators have not determined whether they are precursors of adenocarcinoma.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies in the offspring, including heart defects and limb reduction defects. One case control study estimated a 4.7 fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. OGEN has not been studied for these uses, and therefore should not be used during pregnancy. There is no evidence from well controlled studies that progestogens are effective for these uses.

If OGEN (estropipate tablets) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the question of continuation of the pregnancy should be addressed.

INDICATIONS AND USAGE

The cyclic administration of OGEN (estropipate tablets) is indicated for the treatment of estrogen deficiency associated with (See "DOSAGE AND ADMINISTRATION" section):

1. Moderate to severe *vasomotor* symptoms of menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.)
2. Atrophic vaginitis.
3. Kraurosis vulvae.
4. Female hypogonadism.
5. Female castration.
6. Primary ovarian failure.

OGEN (ESTROPIPATE TABLETS) HAS NOT BEEN TESTED FOR EFFICACY FOR ANY PURPOSE DURING PREGNANCY. SINCE ITS EFFECT UPON THE FETUS IS UNKNOWN, IT CANNOT BE RECOMMENDED FOR ANY CONDITION DURING PREGNANCY (SEE BOXED WARNING).

CONTRAINDICATIONS

OGEN should not be used in women with any of the following conditions:

1. Known or suspected cancer of the breast.
2. Known or suspected estrogen-dependent neoplasia.
3. OGEN may cause fetal harm when administered to a pregnant woman. OGEN is contraindicated in women who are or may become pregnant (See Boxed Warning).
4. Undiagnosed abnormal genital bleeding.
5. Active thrombophlebitis or thromboembolic disorders.
6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use.

WARNINGS

1. *Induction of malignant neoplasms.* Long-term continuous administration of natural and synthetic estrogens in certain animal species has been reported by some investigators to increase the frequency of carcinomas of the breast, cervix, vagina, and liver. There is now evidence that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning).

At the present time there is no conclusive evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast. There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer among these women. Therefore, caution should be exercised when administering estrogens to women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. Careful breast examinations should be performed periodically.

2. *Gall bladder disease.* A recent study has reported a 2 to 3-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens, similar to the 2-fold increase previously noted in users of oral contraceptives. In the case of oral contraceptives, the increased risk appeared after two years of use.

3. *Effects similar to those caused by estrogen-progestogen oral contraceptives.* There are several serious adverse effects of oral contraceptives, most of which have not, up to now, been documented as consequences of postmenopausal estrogen therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women. It would be expected that the larger doses of estrogen used to treat postpartum breast engorgement would be more likely to result in these adverse effects, and, in fact, it has been shown that there is an increased risk of thrombosis in women receiving estrogens for postpartum breast engorgement.

a. *Thromboembolic disease.* It is now well established that users of oral contraceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism; it should also be discontinued during periods of prolonged immobilization.

While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found this does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be used in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men to increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

b. *Hepatic adenoma.* Benign hepatic adenomas appear to be associated with the use of oral contraceptives. Although benign, and rare, these may rupture and cause death through intraabdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations but should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has also been reported in women taking estrogen-containing oral contraceptives. The relationship of this malignancy to these drugs is not known at this time.

c. *Elevated blood pressure.* Increased blood pressure is not uncommon in women using oral contraceptives. There is now a report that this may occur with use of estrogens in the menopause and blood pressure should be monitored with estrogen use, especially if high doses are used.

d. *Glucose tolerance.* A worsening of glucose tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen.

4. *Hypercalcemia.* Administration of estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases. If this occurs, the drug should be stopped and appropriate measures taken to reduce the serum calcium level.

PRECAUTIONS

A. General Precautions.

1. A complete medical and family history should be taken prior to the initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolau smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed.
2. Fluid retention — Estrogens may cause some degree of fluid retention. Therefore, patients with conditions such as epilepsy, migraine, and cardiac or renal dysfunction, which might be influenced by this factor, require careful observation.
3. Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc.
4. Oral contraceptives appear to be associated with an increased incidence of mental depression. Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.
5. Preexisting uterine leiomyomata may increase in size during estrogen use.
6. The pathologist should be advised of the patient's use of estrogen therapy when relevant specimens are submitted.
7. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving estrogen-containing oral contraceptive therapy. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated.
8. Estrogens may be poorly metabolized in patients with impaired liver function and they should be administered with caution in such patients.
9. Because estrogens influence the metabolism of calcium and phosphorus, they should be used with caution in patients with metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency.

B. Information for the Patient. See text of Patient Package Insert which appears after PHYSICIAN REFERENCES.

C. Drug Interactions. The concomitant use of any drugs which can induce hepatic microsomal enzymes with estrogens may produce estrogen levels which are lower than would be expected from the dose of estrogen administered.

The use of *broad spectrum antibiotics* which profoundly effect intestinal flora may influence the absorption of steroidal compounds including the estrogens.

Diabetics receiving *insulin* may have increased insulin requirements when receiving estrogens.

Laboratory Test Interference. Certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The following similar changes may be expected with larger doses of estrogen:

a. Increased sulfobromophthalein retention.
b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.
c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.
d. Abnormal glucose tolerance test results.
e. Decreased pregnanediol excretion.
f. Reduced response to metyrapone test.
g. Reduced serum folate concentration.
h. Increased serum triglyceride and phospholipid concentration.

D. Carcinogenesis. Studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods (see Boxed Warning). At the present time there is no conclusive evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast. There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer among these women. (See "WARNINGS" section.)

E. Pregnancy. Pregnancy Category X. See "CONTRAINDICATIONS" section and Boxed Warning.

F. Nursing Mothers. Estrogens have been reported to be excreted in human breast milk. Caution should be exercised when OGEN is administered to a nursing woman.

G. Pediatric Use. Because of the effects of estrogens on epiphyseal closure, they should be used judiciously in young patients in whom bone growth is not complete.

ADVERSE REACTIONS

(See Warnings regarding reports of possible induction of neoplasia, unknown effects upon the fetus, increased incidence of gall bladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.) The following additional adverse reactions in decreasing order of severity within each category have been reported with estrogenic therapy, including oral contraceptives:

1. *Genitourinary system.*
Increase in size of uterine fibromyomata.
Vaginal candidiasis.
Cystitis-like syndrome.
Dysmenorrhea.
Amenorrhea during and after treatment.
Change in cervical eversion and in degree of cervical secretion.
Breakthrough bleeding, spotting, change in menstrual flow.
Premenstrual-like syndrome.
2. *Breast.*
Tenderness, enlargement, secretion.
3. *Gastrointestinal.*
Cholestatic jaundice.
Vomiting, nausea.
Abdominal cramps, bloating.
4. *Skin.*
Hemorrhagic eruption.
Erythema nodosum.
Erythema multiforme.
Hirsutism.
Chloasma or melasma which may persist when drug is discontinued.
Loss of scalp hair.
5. *Eyes.*
Steepening of corneal curvature.
Intolerance to contact lenses.
6. *CNS.*
Chorea.
Mental depression.
Migraine, dizziness, headache.
7. *Miscellaneous.*
Aggravation of porphyria.
Edema.
Reduced carbohydrate tolerance.
Increase or decrease in weight.
Changes in libido.

OVERDOSAGE

Numerous reports of ingestion of large doses of estrogen-containing oral contraceptives by young children indicate that serious ill effects do not occur. Overdosage of estrogen may cause nausea and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION

1. *Given cyclically for short-term use:*

For treatment of moderate to severe *vasomotor* symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (e.g., 3 weeks on and 1 week off).

Attempts to discontinue or taper medication should be made at 3 to 6 month intervals.

Usual dosage ranges:

Vasomotor symptoms —One OGEN .625 (estropipate) Tablet to one OGEN 5 Tablet per day. The lowest dose that will control symptoms should be chosen. If the patient has not menstruated within the last two months or more, cyclic administration is started arbitrarily. If the patient is menstruating, cyclic administration is started on day 5 of bleeding.

Atrophic vaginitis and kraurosis vulvae — One OGEN .625 Tablet to one OGEN 5 Tablet daily, depending upon the tissue response of the individual patient. The lowest dose that will control symptoms should be chosen. Administer cyclically.

2. *Given cyclically:*

Female hypogonadism; female castration; primary ovarian failure.

Usual dosage ranges:

Female hypogonadism — A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. The lowest dose that will control symptoms should be chosen. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the responsiveness of the endometrium. If satisfactory withdrawal bleeding does not occur, an oral progestogen may be given in addition to estrogen during the third week of the cycle.

Female castration and primary ovarian failure —A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. Adjust dosage upward or downward according to severity of symptoms and response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

HOW SUPPLIED

OGEN (estropipate tablets, USP) is supplied as OGEN .625 (0.75 mg estropipate), yellow tablets, **NDC** 0074-3943-04; OGEN 1.25 (1.5 mg estropipate), peach-colored tablets, **NDC** 0074-3946-04; OGEN 2.5 (3 mg estropipate), blue tablets, **NDC** 0074-3951-04; and OGEN 5 (6 mg estropipate), light green tablets, **NDC** 0074-3958-13. Tablets of all four dosage levels are standardized to provide uniform estrone activity and are grooved (Divide-Tab®) to provide dosage flexibility. All tablet sizes of OGEN are available in bottles of 100.

7013807R

References:

1. Whitehead MI, Townsend PT, Pryse-Davies J, et al: Actions of progestins on the morphology and biochemistry of the endometrium of postmenopausal women receiving low-dose estrogen therapy. *Am J Obstet Gynecol* 142:791-795, 1982.
2. Wren BG, Brown LB, Routledge DA: Differential clinical response to oestrogens after menopause. *Med J Aust* 2:329-332, 1982.
3. Lind T, Cameron EC, Hunter WM, et al: A prospective, controlled trial of six forms of hormone replacement therapy given to postmenopausal women. *Br J Obstet Gynaecol* 86(suppl 3):1-29, 1979.

Abbott Laboratories
Abbott Pharmaceuticals, Inc.
North Chicago, Illinois 60064

American Journal of Obstetrics and Gynecology

Founded in 1920

volume 157 *number* 2 AUGUST 1987

CLINICAL SECTION

Clinical Opinion

When does a statistical fact become an ethical imperative?

Richard K. Silver, M.D., and John Minogue, D.Min.

Chicago, Illinois

Trial of labor is a safe and effective management alternative but remains underused in current clinical practice. To explore whether this underuse is justified, decision analysis is developed to compare a trial of labor with elective repeat cesarean section. With the use of available probability estimates and outcome scoring based on a survey of perinatologists, decision analysis identifies trial of labor as superior to elective repeat cesarean section for both the mother and the neonate. Going beyond the simple comparisons of management options and outcomes, the ethical implications of such results are discussed in terms of "utility ethics," "informed consent," and "universal equality." Such considerations suggest that there is a professional (ethical) responsibility to increase the application of trial of labor. (AM J OBSTET GYNECOL 1987;157:229-33.)

Key words: Trial of labor, decision analysis, utilitarianism

Many claim that what separates a "profession" from a job is the adherence to a set of values and ethical standards. If the profession of medicine is to be something more than the application of basically successful techniques, then that something more will be found in the intangibles of commitment to the ethical principles that guide the practitioner in his/her employment of those techniques. This discussion will attempt to assess trial of labor and the professional/ethical implications of its use as compared to repeat cesarean section. The choice of treatment options will be considered in relation to the principles of "utility ethics," "informed consent," and "universal equality."

Vaginal delivery after a previous cesarean section is considered a contemporary practice alternative. Despite numerous publications confirming the efficacy and safety of this approach, as well as support issued from our specialty,[1] trial of labor remains underused in most medical centers. Even if this alternative is offered by obstetricians, a minority of the eligible patients elect a labor trial, and if oxytocin is disallowed,[2] attempts at vaginal delivery may be abandoned prematurely. The presumed safety of cesarean section, the fear of uterine rupture, and patient preference are among the factors that contribute to the selection of elective surgery over trial of labor. By conservative estimate, if trial of labor is offered at a particular institution, at least half of the repeat cesarean sections performed could be avoided.

In clinical terms, decision analysis is used to identify the treatment plan that provides the best outcome for a majority of patients with a particular condition. Once the best plan is discovered, criteria for a standard of practice may be established. The objectivity of this approach (with the use of both probability estimates and a mathematical model) is appealing when compared with conclusions based on intuition, personal experience, or retrospective review. A risk-benefit assessment may be particularly useful when a prospective study of treatment alternatives is unavailable. Ethically, this type of analysis is known as utilitarianism, whose basic criterion is "do that which promotes the most happiness for the most people."

Shy et al.[3] derived probabilities related to trial of labor and repeat cesarean section. With these figures and a hypothetical cohort of eligible patients, they ap-

From the Department of Obstetrics and Gynecology, Northwestern University Medical School.

Reprint requests: Richard K. Silver, M.D., 2650 Ridge Ave., Evanston, IL 60201.

Table I. Outcome probabilities* for repeat cesarean section and trial of labor

Outcome	*Probability*
Maternal death with vaginal delivery	0.00008
Maternal death with RCS	0.0004
Maternal death with uterine rupture	0.02
Perinatal death with elective term RCS	0.005
Perinatal death with iatrogenic preterm RCS	0.085
Perinatal death with intrapartum RCS	0.008
Perinatal death with vaginal delivery	0.013
Perinatal death with uterine rupture	0.119
Preterm delivery with elective RCS	0.054
Antepartum uterine rupture	0.0019
Intrapartum uterine rupture	0.007
Hysterectomy required after uterine rupture	0.72†
Spontaneous labor before elective RCS	0.20
Failed TOL and indicated RCS	0.33

RCS = Repeat cesarean section; TOL = trial of labor.
*Probabilities adapted from Shy et al.[3]
†Probability derived from references 8 to 10.

plied decision analysis and concluded that maternal and perinatal mortality were similar for either treatment alternative. Although this conclusion might argue in favor of trial of labor, some may view the glass as half empty. If there is no difference in outcome as measured in units of mortality, why not continue with the practice of elective repeat cesarean section? The obvious answer is that there is more to this health care issue than risk of death. A critical concern is the contribution of repeat cesarean section to the "epidemic" of abdominal delivery.[4] Prolonged hospitalization and recovery, surgical morbidity, the need for cesarean section in subsequent pregnancies, and additional cost are examples of other important concerns. Decision analysis provides a mechanism for including the effect of these types of outcome variables along with mortality estimates.

Material and methods

Assessment of accurate probabilities and assignment of relative value scores are the two prerequisites for decision analysis. We used the derived probabilities for trial of labor–related outcomes provided by Shy et al.,[3] as summarized in Table I. In order to include measures of morbidity in addition to mortality estimates, we incorporated nine other complications into our decision trees (Table II). To assign utility scores for all complications, relative rankings and deductions were solicited from eight board-eligible or board-certified perinatologists (three female, five male, excluding the author). These physicians were instructed to rank 11 outcomes in descending order of severity and then to deduct up to 100 points for each item (Table II). The most serious outcome would be ranked first and assigned the largest deduction. Their responses were tabulated and the mean score for each outcome was derived. Next, 100 points were arbitrarily awarded to each outcome pathway of the decision tree from which appropriate deductions were taken. For instance, a woman attempting a trial of labor, who suffers a uterine rupture during labor but is delivered of a healthy infant and survives the operation without the need for hysterectomy, would incur the following deductions: 59 points for intrapartum rupture, 19 points for prolonged hospitalization, 18 points for any future cesarean section, 18 points for additional cost, 10 points for failed trial of labor, 6 points for discomfort, and 3 points for inconvenience (total score of 100 to 133 or −33). Utility scores for each pathway are listed on the right of Figs. 1 and 2. In these figures, the probabilities (in parentheses) are multiplied by the utility scores and the products are summed (from right to left) at each branch of the tree. The management alternative with the greatest score has the highest utility (that is, statistically provides the most happiness to the most patients) and is therefore the clinical approach of choice.

Decision analysis of this type is not designed to function as a model for clinical management (for example, a left-to-right reading of the decision tree). Such analysis would require sensitivity testing of every probability and deletion of all insensitive nodes. In contrast, the approach here is validated only at its end point (in this case, the final score).

Comment

Decision analysis. The higher score for trial of labor (67.3) indicates that when it is compared with elective repeat cesarean section (38.8), this alternative provides the best outcome for mother, baby, and society. Admittedly, the assigned utility scores are subjective. Although the rank order may be agreeable to most clinicians (as was the case for the rankings in our survey; Table II, Rank Agreement column), the specific point assignments may vary among physicians, patients, and other health care providers. We chose a physician source for utility scores because it is from a chiefly medical perspective that counseling is accomplished. With the recognition that patient values may be different, relevant nonmedical concerns (anxiety of awaiting labor, discomfort in labor, and disappointment of a failed trial of labor) were also included in the model and assigned utility scores.

Another possible area of disagreement involves the selection and accuracy of assigned probabilities. To address this criticism, sensitivity testing at any probability node can be performed. Two examples are illustrated as follows. A particular hospital experiences only a 50% vaginal delivery rate among their trial of labor patients. Substitution of this rate in Fig. 2 would reduce the total score to 56.5, while the score for repeat cesarean section would remain 38.8. Similarly, if an exaggerated rate of

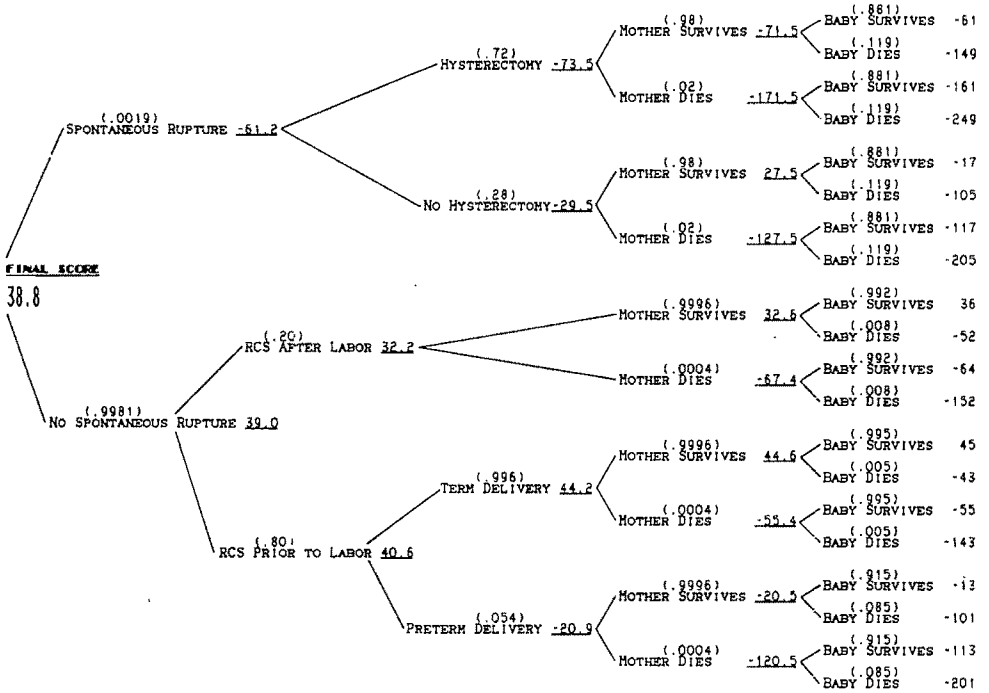


Fig. 1. Decision analysis for repeat cesarean section. Probabilities are in parentheses and subtotal scores are underlined. *RCS* = Repeat cesarean section.

Table II. Assignment of utility scores to maternal and perinatal outcomes

Outcomes listed by rank order	*Mean utility score (± SD)*	*Range*	*Rank agreement**
1. Maternal death	100 ± 0	—	8
2. Neonatal death	88 ± 16	50-100	8
3. Need for hysterectomy	62 ± 16	40-95	7
4. Uterine rupture	59 ± 14	40-80	8
5. Iatrogenic prematurity	58 ± 22	25-90	4
6. Need for future repeat cesarean section	18 ± 10	5-40	6
7. Prolonged hospitalization and recovery	19 ± 10	5-40	5
8. Additional cost	18 ± 8	0-30	7
9. Failed trial of labor	10 ± 6	0-19	7
10. Discomfort of labor	6 ± 9	0-30	7
11. Inconvenience of awaiting labor	3 ± 4	0-10	8

*Number of respondents (out of eight) agreeing with ranking plus or minus one order of rank.

intrapartum uterine rupture were assumed (for example, 5%), the trial of labor score would be reduced to 61.7. In both these instances, the trial of labor score remains higher than its repeat cesarean section counterpart and is therefore the better alternative. In fact, to support the contention that elective repeat cesarean section and trial of labor are equivalent alternatives, one would need to artificially reduce the trial of labor success rate to <25% or increase the intrapartum risk for uterine rupture to >20%.

Even if decision analysis selects trial of labor, wide implementation of this alternative would be difficult in light of the currently recommended trial of labor prerequisites. These include 24-hour blood banking and 30-minute operative start-up time. Admittedly, management of complete uterine dehiscence requires emergency anesthetic services. However, uterine rupture may occur as often before labor as during labor and even occurs among patients without a previous uterine incision. In fact, a recent review by Finley and Gibbs[5] uncovered similar rates of emergent operation for patients with and without a previous cesarean section, implying no greater risk for trial of labor candidates.

Ethical considerations. To shift from numbers to their professional-ethical implications is a fairly straightforward transition if utilitarianism is used. Risk-benefit analysis is born and bred in the American tradition. Using the principles of utility to decide the right

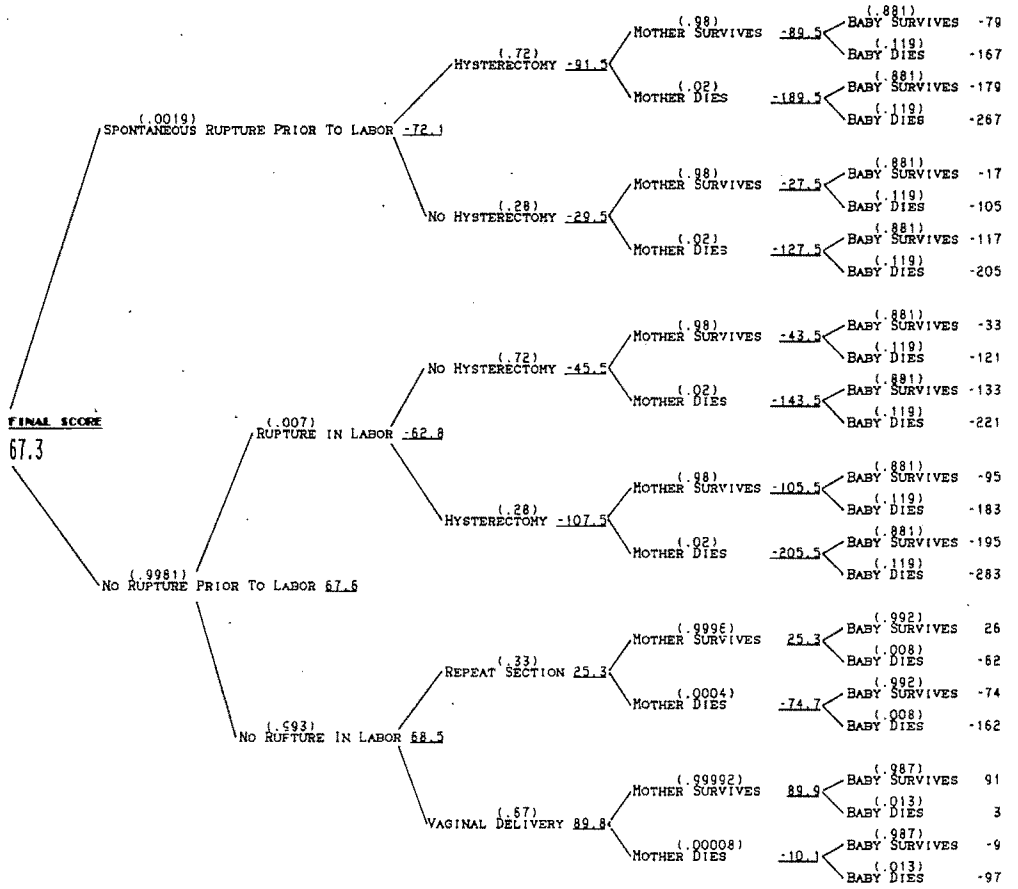


Fig. 2. Decision analysis for trial of labor. Probabilities are in parentheses and subtotal scores are underlined.

and good thing to do runs deep in the American way of thinking; it is also a skill honed to a fine edge in medical training. After all, what is a more congruent way of deciding which course of medical intervention to follow than a risk-benefit analysis? The congruence between the practice of medicine and utilitarianism seems to be a match made in heaven. The development and reliance of modern clinical medicine on statistical analysis of outcome provide utility ethics with the actuarials it needs to derive the outcome-based decision trees. In return, utility ethics provides modern medicine with a license to practice a profession (on the presumption that what distinguishes a profession from a job is a code of ethics that is intrinsic to its functioning).

In regard to the case at hand, using simply utilitarianism, it should be argued that anything other than trial of labor after a previous cesarean section is unethical because the utility scores demonstrate that the trial of labor option holds the best outcome for both the individual patient and the patient population taken as a group. Perhaps the appeal and strength of utilitarian argumentation lie in its focus on outcomes. Although good intentions are nice, for better or worse, it is by results that the medical profession is often judged. What better way to justify a treatment option than to show its positive results.

There are those who are less than euphoric about the relationship between utility ethics and medicine. Two internal problems with the utilitarian system are pointed to: First, it claims objectivity based on a quantification that is really subjective; second, the system has no ability to critically reject any given means, as long as it achieves the most good for the most people. A clinical practice based only on outcome without any commitment to principles would be in danger of subjecting some patients to grave hardship so that most of the patients could benefit. It could easily ignore an equally cherished value and ethical commitment among medical practitioners: the patient's right of autonomy, as safeguarded by the principle of informed consent. The point of these critiques is not to dismiss outcome analysis as a reasonable and ethical criterion for medical decision; it is rather to argue that utilitarianism can neither be the sole criterion of ethical judgment nor be claimed as a purely objective standard. Thus, even though a utility analysis is compelling in regard to trial of labor, perhaps it is not the whole story. The right to

informed consent must be included in any discussion of patient counseling.

Informed consent finds its roots in several considerations. Some would see the principle of informed consent deriving from the simple notion that those who bear the consequences should have the major say in what is to be done. Others see this principle as derived from the very nature of what it is to be human, since to be human is to be responsible and self-determining. Still others would derive the principle of informed consent from a legally granted constitutional right based on social contract. Despite the drastically diverse foundations, each of these reasoning processes would argue that no matter what the outcome, a patient must in principle be informed and consent to a treatment plan. In the extreme, this ethic would argue that the patient has a right to refuse a given treatment, even though such a treatment provides a better outcome than withholding it. This ethic is clearly operative in medicine today.

In relationship to trial of labor, the principle of informed consent would provide that the patient be: (1) accurately informed concerning treatment options, (2) apprised of the specific advantages and disadvantages of trial of labor, and (3) allowed the freedom to determine a reasonable treatment alternative without undue external influence. After being approached to obtain informed consent for trial of labor, 140 of 247 women chose elective repeat cesarean section.[6] Such a low selection rate for a clearly better option by decision analysis might suggest that clear information is not being provided, that inadvertent coercion might be occurring, or that other significant factors influencing patient choice have not been included in our utility model. From the perspective of informed consent, it would be unethical to sway the patients' decisions by use of either unclear information or manipulative images (for example, describing abdominal delivery as a procedure that is safe, convenient, and fairly pain-free while describing labor as long and painful with many uncertainties). Clearly, our own biases in regard to delivery options are going to influence the patients' choices. In fairness to the principle of informed consent, we might make some attempt to eliminate the obvious coercions and balance for the more subtle influences our biases may bring about. Certainly, by the same principle, the patient's concerns must be put into perspective relative to the medical risks of the proposed choices. Specifically, the convenience of a known due date or the avoidance of labor needs to be considered in the context of the risks of elective operation. Although informed consent would not dictate a labor trial, it certainly would be considered unethical if the patient were not presented with correct information in a noncoercive manner.

There are certain types of ethical considerations that are even more restrictive in relation to what options the patient and physician have. Such an ethic is proposed by Immanuel Kant in his book *Fundamental Principles of the Methaphysics of Morals.* His criteria of ethical practice would be derived from the categorical imperative basically stated as: Do only that which you can will to be a universal law.[7] Further, only duty is a fitting motive, for any other motive would be self-serving and less than ethical. Such a system seeks to bring about a universalization of conduct based on principle. Following this type of ethic, if the physician was truly persuaded by the statistics that a trial of labor is best for the patient, then it would be his/her duty to offer only this alternative. This would transcend even the patient's right to choose, since in this system duty and what is right supersede freedom. Though somewhat lockstep, Kant's in-principled ethic has the merit of treating all patients equally and demanding of the physician his/her best effort to ascertain what is best for his/her patient.

In summary, we are obviously persuaded as to the benefit of a labor trial for those patients meeting appropriate guidelines. The arguments presented here favor trial of labor. Not only the numbers but the principles seem to argue for this management option. Certainly what is in the interest of the individual patient is probably best assessed within the personal relationship between the patient and physician. Statistics and general principles cannot overshadow the important variables that make up such relationships. However, as a profession we have an obligation to continually assess, by reasonable standards, what does serve the best interest of our patients.

REFERENCES

1. ACOG Committee on Obstetrics; Maternal-Fetal Medicine. Statement on vaginal birth after cesarean (VBAC). Washington DC: American College of Obstetricians and Gynecologists, 1985.
2. Jarrell MA, Ashmeed GG, Mann LI. Vaginal delivery after cesarean section: a five year study. Obstet Gynecol 1985;65:628-32.
3. Shy KK, LoGerfo JP, Karp L. Evaluation of elective repeat cesarean section as a standard of care: an application of decision analysis. Am J Obstet Gynecol 1981;139: 123-9.
4. Gleicher N. Cesarean section rates in the United States: the short term failure in the National Consensus Development Conference in 1980. JAMA 1984;252:3273-6.
5. Finley BE, Gibbs CE. Emergent cesarean delivery in patients undergoing a trial of labor with a transverse lower segment scar. Am J Obstet Gynecol 1986;155:936-9.
6. Porreco RP. High cesarean section rate: a new perspective. Obstet Gynecol 1985;65:307-11.
7. Munson R. Intervention and reflection: basic issues in medical ethics. 2d ed. Belmont, California: Wadsworth Publishing Company, 1983:14-21.
8. Salzmann B. Rupture of low-segment cesarean section scars. Obstet Gynecol 1963;23:460-6.
9. Donnelly JP, Franzoni KT. Uterine rupture: a 30-year survey. Obstet Gynecol 1963;23:774-7.
10. Eden RD, Parker RT, Gall SA. Rupture of the pregnant uterus: a 53-year review. Obstet Gynecol 1986;68:671-4.

Antithyroid drugs: To breast-feed or not to breast-feed

David S. Cooper, M.D.
Baltimore, Maryland

Historically, women taking antithyroid drugs generally have not been permitted to breast-feed. However, recent studies suggest that infants exposed to the small amounts of antithyroid drugs in breast milk experience no change in thyroid function. Propylthiouracil is the drug of choice in this situation, since it does not cross membranes readily, and milk concentrations are therefore quite low. However, methimazole in low dosages might be used if the infant's thyroid status was monitored at frequent intervals. (Am J Obstet Gynecol 1987;157:234-5.)

Key words: Breast-feeding, propylthiouracil, methimazole

Thyrotoxicosis resulting from Graves' disease occurs commonly enough during pregnancy (one in every 500 to 1000) to warrant periodic review articles[1] and continued research regarding its optimal management.[2, 3] Antithyroid drugs are accepted as the treatment of choice for most patients and have generally been safe and effective therapy for both mother and fetus.[1] However, the question of how to manage the hyperthyroidism after delivery, particularly with respect to breast-feeding, has not been addressed in any comprehensive way in the literature. It is the purpose of this brief review to summarize the available data and formulate specific recommendations regarding antithyroid drugs and the nursing mother.

The problem of antithyroid drugs in breast milk was originally studied by Williams et al.[4] in 1944, with the first antithyroid drug thiouracil. It was demonstrated by a relatively crude nonspecific colorimetric assay that thiouracil concentrations in milk were approximately threefold higher than serum concentrations. Although this drug ceased to be used after 1946, the data on thiouracil levels in milk were extended by inference to include propylthiouracil (introduced in 1946) and methimazole (introduced in 1949). Women taking either of these drugs were advised by most physicians not to breast-feed[5, 6] for fear of causing hypothyroidism in the infant.

The issue was rekindled in 1979 by Low et al.,[7] who administered ^{35}S propylthiouracil to one lactating patient and found that the accumulation of ^{35}S propylthiouracil in milk (measured simply as ^{35}S activity) represented only 0.077% of the administered propylthiouracil dosage over 24 hours. With the subsequent development of sensitive and specific assays for the antithyroid drugs, a number of recent studies have examined this question in more detail. With a sensitive spectrophotometric technique, Kampmann et al.[8] measured propylthiouracil concentrations in the milk of nine lactating women after the oral administration of 400 mg. Only 0.025% of the total dosage was excreted in the milk over 4 hours (i.e., 100 μg). The authors[8] also monitored thyroid function in one infant whose mother was taking 200 to 300 mg propylthiouracil daily and found no change in thyroid function tests, including serum thyroid-stimulating hormone. Since this article, there have been reports[9-11] of a total of six additional infants whose thyroid function tests remained normal while their mothers were taking propylthiouracil in dosages ranging from 125 to 300 mg daily.

Although these limited data on propylthiouracil are reassuring, studies of methimazole (and its close relative carbimazole) are less clear-cut. There are three reports[12-14] involving a total of 10 women, in which methimazole or carbimazole excretion in breast milk was examined. Regardless of the methodology used to measure methimazole, all three studies indicate that methimazole enters the milk freely from the serum compartment, with milk to plasma drug ratios equaling 1. Calculations have been performed showing that an infant might receive as much as 70 μg after a 40 mg dose of methimazole, an amount that could theoretically affect the baby's thyroid function. Nevertheless, Lamberg et al.[9] recently reported on 12 infants whose mothers were treated with the methimazole derivative, carbimazole, in dosages ranging from 5 to 15 mg daily (equal to 3.3 to 10 mg methimazole). All 12 infants had normal thyroid function (including thyroid-stimulating hormone) at 4, 14, and 21 days, and two infants remained normal at 3 and 4 months of age with continued breast-feeding. Thus, at very small dosages, methimazole is probably also a safe drug for the nursing mother.

The theoretical reasons that methimazole but not propylthiouracil would be present in significant concentrations in milk have been reviewed.[10] In brief, the

From the Division of Endocrinology, Sinai Hospital of Baltimore and The Johns Hopkins University School of Medicine.
Reprint requests: David S. Cooper, M.D., Division of Endocrinology, Sinai Hospital of Baltimore, Belvedere and Greenspring Avenues, Baltimore, MD 21215.

most likely explanation is that propylthiouracil is ionized at physiologic pH and is also heavily protein bound (80%), preventing its diffusion across membranes, whereas methimazole is neither ionized nor bound to serum proteins.

The available data suggest that infants exposed to small quantities of antithyroid drugs do not become hypothyroid. On the basis of this assumption, the following recommendations can be made. Any mother who requires antithyroid drug therapy in the postpartum period and who wishes to breast-feed should be allowed to do so. However, the potential risk of neonatal hypothyroidism exists, and the necessity for monitoring the neonate's thyroid function should be made clear to the mother; a serum thyroxine and thyroid stimulating hormone determination every 2 to 4 weeks would be reasonable. Although it has not been reported, the possibility that the infant could have an idiosyncratic reaction (e.g., fever, rash, agranulocytosis) to the drug should also be noted. Although infants have done well after exposure to both propylthiouracil and small dosages of methimazole, propylthiouracil remains the drug of choice because of its lower potency and lower breast milk concentration. However, if the mother were allergic to propylthiouracil, methimazole in dosages of $\leq$10 mg might be used if the infant's thyroid functions were monitored more intensively, at weekly or biweekly intervals.

REFERENCES

1. Burrow GN. The management of thyrotoxicosis in pregnancy. N Engl J Med 1985;313:562-5.
2. Gardner DF, Cruikshank DP, Hays PM, Cooper DS. Pharmacology of propylthiouracil (PTU) in pregnant hyperthyroid women: correlation of maternal PTU concentrations with cord serum thyroid function tests. J Clin Endocrinol Metab 1986;62:217-20.
3. Momotani N, Noh J, Oyanagi H, Ishikawa N, Ito K. Antithyroid drug therapy for Graves' disease during pregnancy: optimal regimen for fetal thyroid status. N Engl J Med 1986;315:24-8.
4. Williams RH, Kay GA, Jandorf BJ. Thiouracil. Its absorption, distribution, and excretion. J Clin Invest 1944; 23:613-27.
5. Ingbar SH, Woebar KA. The thyroid gland. In: Williams RH, ed. Textbook of endocrinology. 6th ed. Philadelphia: WB Saunders, 1981.
6. Spaulding SW, Utiger RD. The thyroid: physiology, hyperthyroidism, hypothyroidism and the painful thyroid. In: Felig P, Baxter JD, Broadus AE, et al eds. Endocrinology and Metabolism. New York: McGraw-Hill, 1981.
7. Low LCK, Lang J, Alexander WD. Excretion of carbimazole and propylthiouracil in breast milk. Lancet 1979; 4:1011.
8. Kampmann JP, Johansen K, Hansen JM, Helweg J. Propylthiouracil in human milk. Revision of a dogma. Lancet 1980;1:736-7.
9. Lamberg BA, Ikonen E, Osterlund K, et al. Antithyroid treatment of maternal hyperthyroidism during lactation. Clin Endocrinol 1984;21:81-7.
10. Cooper DS. Antithyroid drugs. N Engl J Med 1984; 311:1353-62.
11. McDougall IR, Bayer MF. Should a woman taking propylthiouracil breast-feed? Clin Nucl Med 1986;11:249-50.
12. Tegler L, Lindstrom B. Antithyroid drugs in milk. Lancet 1980;2:591.
13. Johansen K, Andersen AN, Kampmann JP, Hansen JM, Mortensen HB. Excretion of methimazole in human milk. Eur J Clin Pharmacol 1982;23:339-41.
14. Cooper DS, Bode HH, Nath B, Saxe V, Maloof F, Ridgway EC. Methimazole pharmacology in man: studies using a newly developed radioimmunoassay for methimazole. J Clin Endocrinol Metab 1984;58:473-9.

The use of fetal biophysical profile improves pregnancy outcome in premature rupture of the membranes

Anthony M. Vintzileos, M.D., Roy Bors-Koefoed, M.D., James F. Pelegano, M.D., Winston A. Campbell, M.D., John F. Rodis, M.D., David J. Nochimson, M.D., and Vasilios G. Kontopoulos, M.D.

Farmington, Connecticut

A protocol using daily fetal biophysical assessment was applied in 73 consecutive patients with premature rupture of the membranes and no clinical signs of infection or labor. A persistently low biophysical score (7 or less on two examinations 2 hours apart) in the presence of a nonreactive nonstress test and absence of fetal breathing was used as an indication for delivery. Measures of pregnancy outcome included the presence of amnionitis or neonatal infection. The outcome of pregnancy in this group of patients was compared with the outcomes of two historic groups: one managed conservatively (control group) and the other managed with amniocentesis on admission to the hospital (amniocentesis group). Infection outcome, maternal as well as neonatal, and low 5-minute Apgar scores were significantly less in the study than in the control group. The frequency of neonatal sepsis was significantly less in the study than in the amniocentesis group. These data suggest that management of premature rupture of the membranes with daily fetal biophysical profiles improves pregnancy outcome by reducing the incidence of maternal and neonatal infection. (Am J Obstet Gynecol 1987;157:236-40.)

The fetal biophysical profile (nonstress test, fetal breathing movements, fetal movements, fetal tone, amniotic fluid volume, and placental grading) has been described previously by us as an early predictor of fetal infection in patients with premature rupture of the membranes and no clinical signs of infection or labor. In that report patients whose last biophysical assessment was within 24 hours of delivery had an infection rate of 93.7% when the biophysical profile score was 7 or less, whereas the frequency of infection with a biophysical profile score of 8 or more was only 2.7%; in that study the fetal biophysical profile results were not used for management.[1] Subsequently, in another report the authors described the efficacy of a protocol using amniocentesis on admission in the management of patients with premature rupture of the membranes; this protocol was found to reduce the frequency of clinical amnionitis and low 5-minute Apgar scores but did not reduce the frequency of neonatal infection, low 1-minute Apgar scores, and perinatal death rate.[2]

The purpose of the present study was to evaluate the efficacy of a protocol using daily fetal biophysical profile assessment in the management of patients with premature rupture of the membranes and no clinical signs of infection or labor. The pregnancy outcome of the study group, as determined by the development of maternal or neonatal infection, was compared with the outcomes of the two historic groups: the first managed conservatively (control group) and the second managed with amniocentesis on admission to the hospital (amniocentesis group). Measures of outcome of infection included the presence of clinical amnionitis, possible neonatal sepsis, and neonatal sepsis.

Material and methods

The study group included 73 consecutive patients with premature spontaneous rupture of the membranes. The majority of patients (71 of 73) were transferred to the University of Connecticut Health Center because of preterm premature rupture of the membranes. The study group consisted of patients admitted to the hospital during the period of August 1, 1985,

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, and the Division of Neonatology, Department of Pediatrics, University of Connecticut Health Center.

Received for publication February 21, 1987; accepted April 27, 1987.

Reprint requests: Anthony M. Vintzileos, M.D., Division of Maternal-Fetal Medicine, University of Connecticut Health Center, Farmington, CT 06032.

Table I. Characteristics of the three groups

	Control group (N = 73)	*p Value*	*Study group (N = 73)*	*p Value*	*Amniocentesis group (N = 73)*
Gestational age (mean ± 2 SD)	31.9 ± 6.1	NS	31.4 ± 5.5	NS	31.0 ± 5.1
Preterm (<37 wk)	69 (94.5%)	NS	72 (98.6%)	NS	71 (97.2%)
Corticosteroids	60 (82%)	NS	62 (85%)	NS	61 (83.5%)
Amniotic fluid					
AF 2	40 (54.7%)	NS	41 (56.1%)	NS	43 (58.9%)
AF 1	16 (21.9%)	NS	14 (19.1%)	NS	12 (16.4%)
AF 0	17 (23.2%)	NS	18 (24.6%)	NS	18 (24.6%)
Birth weight (mean ± 2 SD)	1874 ± 1150 gm	NS	1768 ± 976 gm	NS	1786 ± 1147 gm
No. of fetuses with score ≤7	18 (24.6%)	NS	21 (28.7%)	—	—

AF 2, amniotic fluid pocket ≥2 cm in vertical diameter; AF 1, amniotic fluid pocket 1 to 2 cm in greatest vertical diameter; AF 0, amniotic fluid pocket <1 cm in greatest vertical diameter.

through November 15, 1986. The historic control group (73 patients) consisted of patients with premature rupture of the membranes admitted during the period of July 1, 1983, through June 30, 1984,[1] and the amniocentesis group (73 patients) involved patients admitted consecutively during the period of July 1, 1984, through April 30, 1985.[2] All three groups involved singleton pregnancies and gestational ages of 25 weeks or greater with premature rupture of the membranes and no labor. Patients with signs of labor, chorioamnionitis, bleeding, or fetal distress on admission were excluded. The prior use of tocolysis and the presence of other complicating factors (such as pregnancy-induced hypertension, intrauterine growth retardation, and diabetes mellitus) were also exclusion criteria. The inclusion and exclusion criteria were the same for the three groups.

In all patients, rupture of the membranes was diagnosed by sterile speculum examination with pooled fluid, ferning, and alkaline pH determination (Nitrazine Paper). Bimanual pelvic examinations were not performed unless the patients were believed to be in active labor or a decision had been made to induce labor. On admission to the hospital, maternal vital signs were recorded and electronic fetal heart rate monitoring was used to detect signs of fetal distress and document any uterine activity. If there was no evidence of labor, fetal distress, or infection, an ultrasonographic evaluation was performed for confirmation of the gestational age and determination of the fetal biophysical profile. The ultrasonographic evaluation was performed by means of a linear-array real-time ultrasonographic method (General Electric SRT 2600, Wilmington, Massachusetts) equipped with a 3.5 MHz transducer. Real-time scanning consisted of a maximum 30-minute observation period during which fetal movements and fetal breathing movements were counted and fetal tone, amniotic fluid volume, and placental grading were estimated. Each biophysical variable was scored as 2, 1, or 0 according to the criteria cited in a previous study.[3] The maximal score was 12 and the minimal 0. In the historic control group the results of the fetal biophysical profile were not used for management. In the amniocentesis group immediately after the ultrasonographic examination, an amniocentesis under direct ultrasonic guidance was performed and the fluid was sent immediately for Gram staining and cultures. In the study group an abnormal biophysical assessment was used as an indication for delivery (biophysical score of 7 or less on two examinations 2 hours apart in the presence of a nonreactive nonstress test and absence of fetal breathing).

All three groups were observed in the hospital and were managed conservatively with bed rest and bathroom privileges. Corticosteroid therapy was administered in the majority of patients (Table I). Temperatures were taken four times a day and white blood cell counts were performed daily. Antibiotics were administered to the mother only after the cord was clamped. In the study group the fetal biophysical profile determinations were repeated every 24 hours if patients remained undelivered. Indications for delivery in the control group included labor, clinical amnionitis, persistent spontaneous variable decelerations (moderate to severe), or gestational age >37 weeks after a 24-hour period of observation. The results of the fetal biophysical profile were not used in the management of the control group. Indications for delivery in the amniocentesis group were the same as for the control group in addition to a positive Gram stain or culture of the obtained amniotic fluid and a lecithin:sphingomyelin ratio of 2 or greater. Indications for delivery in the study group were the same as for the control group in addition to a persistently low fetal biophysical score

Table II. Comparison of outcome of pregnancy between the three groups

	Control group (N = 73)	*p Value*	*Study group (N = 73)*	*p Value*	*Amniocentesis group (N = 73)*
Overall infectious outcome*	22 (30.1%)	<0.005	8 (10.9%)	NS	16 (21.9%)
Amnionitis	15 (20 5%)	<0.01	4 (5.4%)	NS	5 (6.8%)
Possible neonatal sepsis	10 (13 6%)	NS	4 (5.4%)	NS	9 (12.3%)
Neonatal sepsis	7 (9 5%)	NS	1 (1.3%)	<0.05	9 (12.3%)
Overall neonatal infection†	17 (23.2%)	<0.005	5 (6.7%)	<0.005	18 (24.6%)
Apgar score <7					
1 min	23 (31.5%)	NS	17 (23.2%)	NS	26 (35.6%)
5 min	9 (12.3%)	<0.005	0 (0.0%)	NS	2 (2.7%)
Umbilical cord pH (mean ± 2 SD)					
Artery	7.29 ± 0.12	NS	7.28 ± 0.16	NS	7.28 ± 0.12
Vein	7.34 ± 0.11	NS	7.35 ± 0.14	NS	7.34 ± 0.10
Perinatal deaths	2 (2.7%)	NS	1 (1.3%)	NS	1 (1.3%)

*Overall infectious outcome includes patients with infection (mother or neonate), some of whom had more than one diagnosis.
†Overall neonatal infection combines neonates with possible neonatal sepsis and neonatal sepsis.

(7 or less on two examinations 2 hours apart in the presence of a nonreactive nonstress test and absence of fetal breathing).

On admission to the neonatal intensive care unit, cultures were performed in all infants (blood, urine, cerebrospinal fluid, trachea, nasogastric, and external ear). All neonates received prophylactic antibiotics that were discontinued when the cultures demonstrated no growth. When neonatal sepsis or possible neonatal sepsis was diagnosed, the antibiotics were continued for approximately 7 to 10 days. The diagnosis of clinical amnionitis, possible neonatal sepsis, and neonatal sepsis was based on previously described criteria.[1-4] The pregnancy outcome of the study group, as determined by the development of maternal or neonatal infection, was compared with the outcomes of the two historic groups: the first managed conservatively (control group) and the second managed with amniocentesis on admission (amniocentesis group). Postdelivery data collection included Apgar scores, cord blood pH (umbilical artery and vein), neonatal septic workup, and perinatal deaths.

Statistical analysis was performed by the χ^2, Fisher's exact, or Student t test when appropriate.

Results

The study group included 73 consecutive patients with premature rupture of the membranes. A total of 231 fetal biophysical profiles were done in these 73 patients (3.1 per patient). Twenty examinations were the most performed on one patient. The mean gestational age on admission to the hospital was 31.4 weeks (range 25 to 38 weeks) and the mean birth weight (±2 SDs) was 1768 gm (±976 gm). The cesarean section rate was 48% (35 of 73).

The characteristics of the three groups on admission are shown in Table I. There were no differences between the three groups in terms of mean gestational age, preterm gestations, corticosteroid administration, birth weight, and distribution of amniotic fluid volume. Because the degree of oligohydramnios has been described as a risk factor for infectious morbidity in patients with premature rupture of the membranes,[4,5] all three groups were also compared according to amniotic fluid distribution but no differences were found. The number of fetuses with low biophysical scores (7 or less) was not different between the study and control group.

The comparison of outcomes of pregnancy between the three groups is shown in Table II. Compared with the control group, the study group was found to have a significantly lower frequency of overall infectious outcome (number of patients who developed infection; $p < 0.005$), amnionitis ($p < 0.01$), and 5-mminute Apgar scores ($p < 0.005$). The p values for possible neonatal sepsis and neonatal sepsis were 0.09 and 0.06, respectively. When possible neonatal sepsis and neonatal sepsis were combined (overall neonatal infection) the difference became statistically significant ($p < 0.005$). There were no differences between the study and control groups in terms of 1-minute Apgar scores <7, umbilical cord pH (artery and vein), and perinatal death rate. Compared with the amniocentesis group, the study group was found to have a significantly lower frequency of neonatal sepsis ($p < 0.05$). The overall neonatal infection was also significantly less in the study group ($p < 0.005$). There were no differences between

the study and amniocentesis groups in terms of overall infectious outcome (number of patients who developed infection), amnionitis, possible neonatal sepsis, Apgar scores, umbilical cord pH (artery and vein), and perinatal death rate.

Eight (10.9%) patients developed infection in the study group. The diagnoses were clinical amnionitis (four cases), possible neonatal sepsis (four cases), and neonatal sepsis (one case). One patient had more than one diagnosis. Of the eight patients with infection, delivery was accomplished in seven because of low biophysical score, four of whom developed intrapartum amnionitis, and in one because of spontaneous onset of labor. Of the remaining 65 patients with no infection, delivery was accomplished in 41 because of spontaneous onset of labor, in 14 because of low biophysical score (7 or less), in seven because of moderate to severe variable decelerations on electronic fetal heart rate monitoring, and in three because of suspected abruptio placentae. There was one false negative biophysical profile assessment in a fetus born vaginally who went on to develop possible neonatal sepsis. There was one neonatal death (1.3%) in the population studied; this fetus was 26 weeks' gestation and was delivered by cesarean section because of a low biophysical score. The infant died of disseminated sepsis and prematurity.

Comment

The fetal biophysical profile has been described recently as an early predictor of fetal infection in patients with premature rupture of the membranes.[1] In that report, which involved the use of frequent fetal biophysical profile determinations, the individual biophysical variables—nonstress test, fetal breathing movements, fetal movements, and fetal tone—were found to be important in predicting infection outcome in the above sequence; however, the best predictor of outcome of infection was found to be the overall biophysical score. The first manifestations of fetal infection were nonreactive nonstress testing and loss of fetal breathing. The loss of fetal movement and fetal tone were late signs of infection. That study was only observational and the results of the fetal biophysical assessment were not used for management. These patients formed the control group of the present study. Based on the results of that study the authors formulated a protocol for following patients with premature rupture of the membranes based on daily fetal biophysical profile determinations.

The purpose of the present study was to evaluate the efficacy of this protocol using daily fetal biophysical assessment in the management of patients with premature rupture of membranes and to see if such an approach would improve the outcome of pregnancy in these patients. The outcome of pregnancy of the study group was also compared with another historic group (amniocentesis group) managed by a protocol of transabdominal amniocentesis on admission to the hospital; the results of such a protocol have been reported previously by our group.[2] All three groups were similar in terms of risk factors for development of infection (Table I).

The present data suggest that management of premature rupture of the membranes with daily fetal biophysical assessment reduces the frequency of maternal and neonatal infection. The percentage of patients who developed infection in the study group (10.9%) was significantly less than in the control group (30.1%). This represents a reduction in our infection rate by almost two thirds. In the control group 18 patients had a low biophysical score and 16 (89%) developed infection. However, that study was observational and delivery was not undertaken in patients with low biophysical scores. In the majority of these 16 patients with infection (10 of 16), delivery occurred more than 2 days after the first abnormal fetal biophysical assessment. In the present study there were 21 fetuses with low biophysical scores; however, immediate action and prompt delivery was undertaken in all cases based on the abnormal biophysical assessment. This resulted in an overall infectious outcome (number of patients with infection) of 10.9%. The incidence of possible neonatal sepsis and neonatal sepsis were also lower in the study group compared with the control group (5.4% versus 13.6% and 1.3% versus 9.5%), but possibly because of the small numbers this did not reach statistical significance. However, when combined possible neonatal sepsis and neonatal sepsis were considered together, the overall neonatal infection was significantly lower in the study group (6.7% versus 23.2%). These data support the findings of our earliest studies[1,6] in which we have reported that the fetal biophysical profile is a good predictor of fetal infectious compromise. As to the significance of the higher 5-minute Apgar scores in the study group, one speculation is that it might be a reflection of the lower incidence of infection in the study population at the time of delivery. A higher incidence of low 5-minute Apgar scores given to infants born of mothers with clinical infection has been reported previously.[1,2]

Compared with the amniocentesis group the study group was found to have a significantly lower frequency of neonatal sepsis and overall neonatal infection. This is in agreement with our previously reported experience[2] with transabdominal amniocentesis, which has shown that this protocol decreases the incidence of clinical amnionitis but does not improve the outcome of neonatal infection. The outcome of neonatal infection was reduced by fetal biophysical assessment, a modality

that reflects fetal status rather than the presence of intrauterine bacteria.

A prospective, randomized study would be the ideal design to address the question of whether management of premature rupture of the membranes with daily fetal biophysical assessment improves the outcome of pregnancy. Although scientifically desirable, such a study was ethically unacceptable to all maternal-fetal medicine subspecialists and neonatologists at our institution. Therefore we used historic groups for comparison. We feel that the similarity between the historic and study groups permitted a meaningful clinical comparison because all three groups consisted of consecutive patients who were studied prospectively and managed in a similar manner other than the indications for delivery.

Our protocol for following patients with preterm premature rupture of the membranes takes into consideration the condition of the fetus before deciding on the optimal plan of management. Our data suggest that this approach improves the outcome of pregnancy by reducing the incidence of maternal and neonatal infection, although in the current study it did not improve the perinatal death rate.

REFERENCES

1. Vintzileos AM, Campbell WA, Nochimson DJ, et al. The fetal biophysical profile in patients with premature rupture of the membranes—an early predictor of fetal infection. Am J Obstet Gynecol 1985;152:510.
2. Feinstein SJ, Vintzileos AM, Lodeiro JG, et al. Amniocentesis with premature rupture of membranes. Obstet Gynecol 1986;68:147.
3. Vintzileos AM, Campbell WA, Ingardia CJ, et al. The fetal biophysical profile and its predictive value. Obstet Gynecol 1983;62:271.
4. Vintzileos AM, Campbell WA, Nochimson DJ, et al. Degree of oligohydramnios and pregnancy outcome in patients with premature rupture of the membranes. Obstet Gynecol 1985;66:162.
5. Gonik B, Bottoms SF, Cotton DB. Amniotic fluid volume as a risk factor in preterm premature rupture of the membranes. Obstet Gynecol 1985;65:456.
6. Vintzileos AM, Campbell WA, Nochimson DJ, et al. Fetal biophysical profile versus amniocentesis in predicting infection in preterm premature rupture of the membranes. Obstet Gynecol 1986;68:488.

The effects of maternal ambulation on the nonstress test

Lawrence D. Devoe, M.D., Martha Arthur, B.S.N., and Nancy Searle, B.A.

Augusta, Georgia

Using a recently developed noninvasive telemetry device (model 8240A, Hewlett-Packard Co., Palo Alto, California), we studied, under controlled conditions, the effects of maternal ambulation on the nonstress test patterns of 100 near-term high-risk pregnancies, randomly divided into two equal groups who underwent three alternating 30-minute periods of ambulation and bed rest, beginning either with ambulation (group 1) or bed rest (group 2). Fetal heart rate data were captured and analyzed by a programmed microcomputer for group mean 30-minute fetal heart rate baseline rates and variability and incidences and dimensions of fetal heart rate accelerations and decelerations. Both groups were similar for the maternal and perinatal data examined. The fetal heart rate parameters analyzed were similar within and between each group during each 30-minute testing segment. No episodes of sustained tachycardia or bradycardia were observed. Although 13 fetuses in each group exhibited variable or late decelerations, the incidence of decelerations was no different during periods of rest or ambulation. Maternal ambulation does not elicit abnormal fetal heart rate responses in most high-risk fetuses and may be an acceptable alternative to conventional nonstress test conditions. (Am J Obstet Gynecol 1987;157:240-4.)

Key words: Maternal ambulation, nonstress test, fetal heart rate

From the Department of Obstetrics and Gynecology, Medical College of Georgia.
Supported in part by the Hewlett-Packard Company, Andover, Massachusetts.
Received for publication September 15, 1986; revised January 13, 1987; accepted March 19, 1987.
Reprint requests: Lawrence D. Devoe, M.D., Maternal-Fetal Medicine Section, Department of Obstetrics and Gynecology, Medical College of Georgia, Augusta, GA 30912.

The nonstress test, an accepted method of fetal evaluation,[1] is usually performed with the patient resting in a semirecumbent or lateral position. Such maternal testing conditions generate legible fetal heart rate tracings but do not reflect the extrauterine environment that the fetus encounters during normal daily levels of maternal activity. Earlier studies of fetal heart rate re-

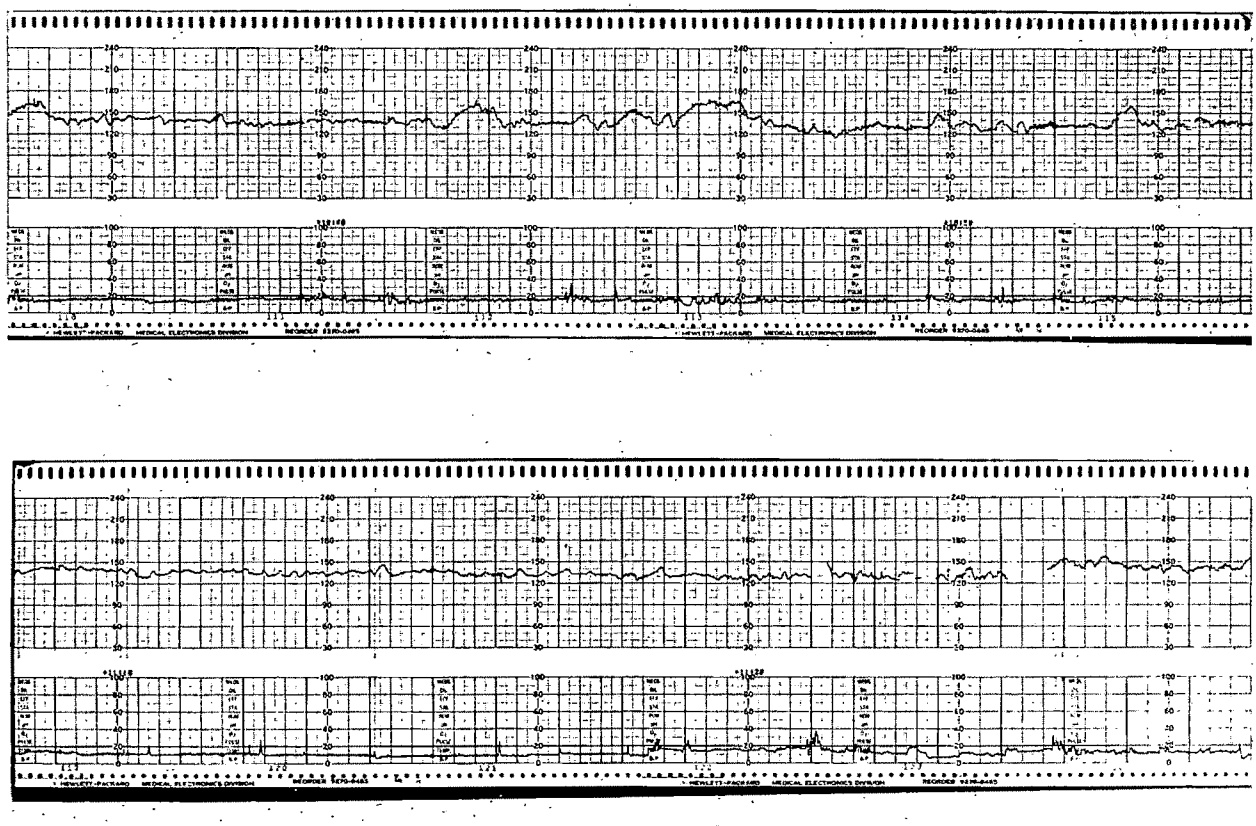


Fig. 1. *Upper panel* shows a fetal heart rate tracing obtained by telemetry during ambulation, and *lower panel* shows a fetal heart rate tracing obtained by telemetry during bed rest.

sponses to strenuous maternal activity[2-5] used relatively "noisy" fetal heart rate transducer systems, were performed on relatively small groups, or often contained discontinuous recordings of fetal heart rate patterns during the actual periods of maternal exercise. These studies did not consistently account for other testing conditions such as maternal fed state or time of day, and standard lengths of observation were not used. Subsequent investigations,[6-12] using more sophisticated systems to study fetal heart rate response to constant or graded levels of maternal exercise, have also not considered all of the above conditions associated with fetal heart rate testing. A variety of fetal heart rate responses have been observed during or after maternal exercise, including sustained tachycardia or bradycardia, with and without loss of baseline variability, as well as no change in baseline fetal heart rate. Newer noninvasive fetal heart rate telemetry units that produce high-quality Doppler fetal heart rate recordings have encouraged the present study of the effects of maternal ambulation on several fetal heart rate parameters obtained during nonstress testing (i.e., baseline fetal heart rate and its long-term variability and the frequency and dimensions of fetal heart rate accelerations and decelerations). In addition, we wished to determine whether maternal ambulation altered the time needed to achieve nonstress test reactivity and was a clinically practicable alternative to conventional testing conditions.

Material and methods

One hundred pregnant women were enrolled from the Obstetrical Clinic of the Medical College of Georgia. All met the following criteria: (1) gestational age established by menstrual data and ultrasonographic corroboration; (2) singleton fetus without anomalies; (3) established high-risk conditions for antepartum fetal testing; (4) intact amniotic membranes; and (5) no medical or physical limitations for ambulation.

Testing was performed under a standard maternal state protocol[13] that included test initiation between 8:30 AM and 1 PM, 2 hours postprandially, with the avoidance of smoking or other drugs that might affect the fetal cardiovascular or central nervous system. Fetal heart rate and tokodynamometric signals were transmitted to a model 8040A electronic monitor (Hewlett-Packard Co., Palo Alto, California) in the Doppler mode, by a noninvasive telemetry device (Hewlett-Packard model 8240A). These data were captured online by an IBM microcomputer, interfaced with the electronic monitor, which used a software program

Table I. Indications for antepartum fetal heart rate testing

Diagnosis	*Group 1**	*Group 2**
Intrauterine growth retardation	21	17
Postdates	13	11
Diabetes mellitus	3	4
Hypertension	4	4
Decreased fetal movement	5	8
Decreased amniotic fluid volume	2	2
Miscellaneous	2	4
Total	50	50

$\chi^2 = 2.43$, $p > 0.9$.
*See text for group descriptions.

developed and validated at the Medical College of Georgia.

Each study session lasted 90 minutes. Before testing the patients were randomly assigned to one of two groups of equal size. Patient in group 1 began their nonstress tests during ambulation for 30 minutes, followed by 30 minutes of bed rest and an additional 30 minutes of ambulation. For the nonstress tests of patients in group 2 the order of ambulatory and resting sessions was reversed. After a 60-second baseline recording of resting maternal heart rate, the patients walked in the fetal testing unit at a pace deemed similar to their normal pace at home. At the end of each ambulation period, maternal heart rate was remeasured. Ambulation took place at distances of 10 to 50 feet from the telemetry receiving unit. No study was discontinued for either fetal or maternal indications. Fig. 1 illustrates a typical fetal heart rate tracing obtained during ambulation *(upper panel)* and during bed rest *(lower panel)* in a patient monitored with the telemetry device.

Computer analysis generated 30-minute segments of mean baseline fetal heart rate, mean heart rate variation (coefficient of variation of mean beat-to-beat differences averaged each minute), signal loss, mean frequency, amplitude and duration of fetal heart rate accelerations exceeding 10 and 15 bpm, and the frequency and dimensions of fetal heart rate decelerations if present. Statistical comparisons of these fetal heart rate parameters were made for mean 30-minute testing sessions within and between each group. Other data analyzed included maternal age and parity, gestational age at delivery, test-to-delivery interval, birth weight, Apgar score at 5 minutes, method of delivery, and the presence of intrapartum or neonatal complications. Statistical methods included multiple *t* tests, analysis of variance, or χ^2 tests where appropriate.

Results

Maternal and perinatal outcomes. Both study groups were similar for the specific high-risk test indications as shown in Table I. Table II demonstrates that both groups were also similar for maternal gravidity and parity, gestational age at testing, test-to-delivery interval, birth weight, cesarean section rates for intrapartum fetal distress, and 5-minute Apgar scores. The actual incidences of intrauterine growth retardation, defined as birth weight below the tenth percentile for gestational age, and postmaturity syndrome were also similar for both groups (Table II). There was one neonatal death in group 2 of a 2497 gm male infant, born at 33 weeks' gestational age to a mother with class D diabetes mellitus. Death was attributed to the sequelae of profound intrapartum asphyxia, resulting from abruptio placentae.

Fetal and maternal heart rate parameters during ambulatory and nonambulatory testing sessions. Within each group, mean maternal pulse rate after ambulation rose by an average of 20.5 ± 4.5 (SD) bpm over mean resting pulse rates and did not differ significantly for any ambulatory session. Mean 30-minute baseline rate or variation and the incidence or dimensions of 10 or 15 bpm accelerations did not differ significantly from one testing session to another within or between the two groups (Table III). The incidence of fetuses with significant variable or late decelerations ranged from 12% to 18% during any 30-minute session and was most frequently associated with postmaturity syndrome (seven cases). Thirteen fetuses in each group exhibited occasional late or variable decelerations during one or more of their 30-minute test segments. The outcomes of these fetuses and their distribution during the testing segments are shown in Table IV. The actual frequency of variable or nonrepetitive late decelerations did not differ during any test period within or between the groups. Neither prolonged tachycardia nor bradycardia was observed during any of the testing sessions. Mean signal loss was significantly higher during maternal ambulation (7.7% ± 6.7% [SD]) than during bed rest (4.8% ± 6.8% [SD]) but did not exceed 22% in any single 30-minute period. The mean time required to achieve nonstress test reactivity (the presence of at least three accelerations exceeding 15 bpm and 15 seconds' duration) was similar whether testing was begun with ambulation (23.8 ± 20.5 minutes) or bed rest (19.7 ± 17.3 minutes). Only one nonstress test in each group failed to become reactive within 90 minutes; in each instance the pregnancy was complicated by intrauterine growth retardation.

Comment

Recent studies[6-12] indicate a renewal of interest in the acute effects of maternal exercise on the fetal cardiovascular system. Most have investigated activity levels more strenuous than those associated with ambulation. Few have maintained continuous fetal heart rate monitoring during the actual period of exercise,[12] and most

Table II. Maternal and perinatal characteristics of the study population

	Group 1	*Group 2*	*p*
Maternal age (yr)	20.7 ± 5.0*	23.1 ± 4.2	<0.01†
Gravidity	1.8 ± 1.3	2.2 ± 1.4	>0.10†
Parity	0.6 ± 1.2	0.7 ± 0.9	>0.10†
Gestational age (yr)	38.2 ± 3.1	38.8 ± 2.2	>0.10†
Test-to-delivery interval (days)	12.3 ± 13.0	9.4 ± 0.9	>0.05†
Birth weight (gm)	3085 ± 469	3083 ± 762	>0.10†
5 min Apgar score	9.0 ± 0.4	8.8 ± 0.9	>0.10†
Complications			
5 min Apgar score <7	2	2	>0.05‡
Intrapartum fetal distress	4	6	>0.05§
Intrauterine growth retardation	3	4	>0.05‡
Perinatal deaths	0	1	0.50§

*Data presented as mean ± SD.
†By *t* test.
‡By χ^2 test.
§Fisher's exact test.

Table III. Fetal heart parameters in ambulatory patients (group 1) and those at bed rest (group 2)

	First 30 min		*Second 30 min*		*Third 30 min*	
	Group 1	*Group 2*	*Group 1*	*Group 2*	*Group 1*	*Group 2*
Baseline fetal heart rate (bpm)	140 ± 8*	137 ± 8	138 ± 7	138 ± 7	137 ± 7	138 ± 11
Minute coefficient of variation	0.035 ± 0.01	0.035 ± 0.01	0.037 ± 0.01	0.039 ± 0.01	0.041 ± 0.02	0.036 ± 0.01
Frequency of accelerations >15 bpm	5.8 ± 4.2	6.7 ± 5.1	6.6 ± 4.8	6.4 ± 4.3	6.0 ± 4.9	6.4 ± 4.8
Frequency of accelerations >10 bpm	10.3 ± 6.2	11.5 ± 6.2	12.5 ± 6.4	12.7 ± 5.4	11.6 ± 6.3	11.2 ± 6.2
Amplitude of accelerations >15 bpm	19.4 ± 2.8	19.9 ± 3.3	20.1 ± 3.6	20.4 ± 5.2	20.6 ± 5.8	20.5 ± 3.7
Amplitude of accelerations >10 bpm	15.1 ± 2.5	6.2 ± 3.8	16.1 ± 2.8	16.4 ± 3.9	16.5 ± 4.0	16.7 ± 4.2
Duration of accelerations >15 bpm (sec)	29.1 ± 16.2	25.5 ± 12.1	27.9 ± 13.5	27.0 ± 12.1	27.7 ± 12.9	29.4 ± 13.7
Duration of accelerations >10 bpm (sec)	21.9 ± 7.6	20.3 ± 9.7	22.1 ± 9.2	23.5 ± 7.1	21.5 ± 9.0	23.9 ± 10.4

*All values expressed as means ± SD.

have confined their observations to preexercise and postexercise effects on fetal heart rate baseline in normal pregnant patients. With few exceptions,[10, 11] factors associated with fetal heart rate testing conditions, such as maternal fed state or time of day, were not specific in these studies. Although the impact of maternal dietary state on fetal activity may be controversial, data reported by Richardson et al.[14] and our group[15] suggest that quantitative differences in fetal biophysical activity patterns may exist when maternal fasting and fed states are compared. Finally, only one previous study has addressed the effects of maternal exercise on fetal heart rate reactivity.[16] This study examined only seven normal subjects, did not use continuous monitoring, and had observation intervals of 60 minutes or less. Therefore it is quite difficult to make valid direct comparisons

Table IV. Incidence and outcomes of fetuses with late or deep variable heart rate decelerations

	Group 1 (N = 13)	*Group 2 (N = 13)*
Test segment*		
First 30 min	7	9
Second 30 min	6	7
Third 30 min	8	7
Complication		
Postmaturity	5	2
Intrapartum fetal distress	4	6
Intrapartum growth retardation	1	1
5 min Apgar score <7	1	1
None	2	3

*The same fetus may be represented in more than one 30-minute segment.

of these previous data with those of the present study.

The general impression suggested by earlier work is that the response of fetal heart rate baseline to maternal exercise, whether mild or moderate, may be quite variable in normal subjects and includes significantly elevated or reduced baseline fetal heart rates, as well as the absence of fetal heart rate baseline changes. The application of ambulatory fetal heart rate monitoring to the detection of fetal compromise in complicated pregnancies has been very limited, involving primarily the observation of changes in baseline fetal heart rate relative to the period of maternal exertion. Although Stembera[2] and Pomerance et al.[4] noted a positive correlation between alteration in baseline fetal heart rate and subsequent fetal outcome, neither report led to widespread clinical use of maternal exercise testing.

The present study differs from previous reports in that all subjects were drawn from a high-risk population. Fetal heart rate monitoring was continuous throughout the observation period, and fetal heart rate parameters were analyzed in addition to baseline rate. It does share a problem common to many of the earlier studies because maternal physiologic parameters other than heart rate were not measured and the rate of ambulation was not rigidly controlled. Therefore it is possible that in spite of similar postambulatory increases in maternal heart rate, the absolute level of exertion was not the same for all ambulatory patients. Our data suggest that maternal ambulation does not significantly alter the fetal heart rate patterns of either normal or compromised fetuses, when compared with those obtained during bed rest. Maternal ambulation is associated with higher loss of fetal heart rate signal and does not shorten the time required to produce a reactive nonstress test result. However, in no instances was fetal heart rate legibility unsatisfactory for visual interpretation. Telemetry devices that can transmit a high-quality signal may allow fetal heart rate testing to be performed in a setting more acceptable to patients who prefer not to be physically confined to a bed or chair. With the future development of sophisticated remote communication systems, this may also provide the potential for performing such testing at home, thus making ambulatory fetal heart rate testing even more convenient for high-risk patients. Whether the cost of this equipment and the inherent trade-off of increased signal loss could make this application more widely applicable requires further investigation.

REFERENCES

1. Rochard F, Schifrin BS, Goupil F, et al. Nonstressed fetal heart rate monitoring in the antepartum period. Am J Obstet Gynecol 1976;126:699.
2. Stembera ZK. Fetal tolerance to maternal exercise hypoxia. In: Perinatal factors affecting human development. Washington, DC: Pan American Health Organization, 1969:105-110.
3. Soiva K, Salmi A, Gronroos M, Pelton T. Physical working capacity during pregnancy and effect of physical tests on fetal heart. Am Chir Gynecol 1983;53:187.
4. Pomerance JJ, Gluck L, Lynch VA. Maternal exercise as a screening test for utero placental insufficiency. Obstet Gynecol 1974;44:383.
5. Hon EH, Wohlgemuth R. The electronic evaluation of fetal heart rate. Am J Obstet Gynecol 1961;81:361.
6. Collings CA, Curet LB, Mullin JP. Maternal and fetal responses to a maternal aerobic exercise program. Am J Obstet Gynecol 1983;145:702.
7. Collings C, Curet LB. Fetal heart rate response to maternal exercise. Am J Obstet Gynecol 1985;131:498.
8. Clapp JF. Fetal heart rate response to running in midpregnancy and late pregnancy. Am J Obstet Gynecol 1985;153:251.
9. Jovanovic L, Kessler A, Peterson CM. Human maternal and fetal response to graded exercise. J Appl Physiol 1985;58:1719.
10. Marsal K, Lofgren O, Gennser G. Fetal breathing movements and maternal exercise. Acta Obstet Gynecol Scand 1979;58:197.
11. Platt LD, Artal R, Semel J, Sipos L, Kammula RK. Exercise in pregnancy: II. Fetal responses. Am J Obstet Gynecol 1983;147:487.
12. Artal R. Fetal responses to maternal exercise. In: Artal R, Wiswell RA, eds. Exercise in pregnancy. Baltimore: William & Wilkins, 1985:195.
13. Devoe LD, McKenzie J, Searle NS, Sherline DM. Clinical sequelae of the extended nonstress test. Am J Obstet Gynecol 1985;151:1075.
14. Richardson B, Briggs ML, Toomey C, Burry KJ, O'Grady JP. The effect of maternal glucose administration on the specificity of the nonstress test. Am J Obstet Gynecol 1983;145:141.
15. Devoe LD, Searle N, Castillo RA, Searle J. Fetal biophysical testing: the effects of prolonged maternal fasting and the oral glucose tolerance test. J Reprod Med (in press).
16. Hauth JC, Gilstrap LC, Widmer K. Fetal heart rate reactivity before and after maternal jogging during the third trimesters. Am J Obstet Gynecol 1982;142:545.

Fetal monitoring during emergency obstetric transport

John P. Elliott, M.D., and Rebecca Trujillo, R.N.
Phoenix, Arizona

The practicality of fetal heart rate monitoring during fixed-wing transport of obstetric emergencies was evaluated. Fifty-seven transports were performed and 40 (70%) were monitored successfully with the electronic fetal heart rate monitor. External fetal heart rate monitoring was technically accomplished in 83% of monitored transports. Of the 40 patients monitored, 33 (83%) of the fetal heart rate tracings were considered useful for adequate evaluation of the fetal heart rate and contraction frequency. No patients had late decelerations during transport. At cabin pressures from 1100 feet to 7000 feet, fetal hypoxia as measured by late decelerations was not present in any patient. (Am J Obstet Gynecol 1987:157:245-7.)

Key words: Fetal heart rate, maternal transport, monitoring, fetal distress

Maternal transport of high-risk perinatal patients has resulted in improved survival, decreased short- and long-term morbidity, and decreased cost of hospitalization for the infants transported in utero and delivered at a tertiary care center.[1, 2] Short-distance transports can be accomplished by ambulance or helicopter, but longer-distance transports are usually accomplished with fixed-wing aircraft. The safety of travel for the fetus at altitude has not been adequately established as evidenced by different policies regarding flying while pregnant for transport nurses, flight attendants, and pilots.

Adequate assessment of the fetus in a high-risk situation would ideally use the technique that gives the most accurate information. Electronic fetal heart rate monitoring has become the accepted standard of care for intrapartum monitoring of high-risk pregnancies. Many of the transported patients are in labor and in a high-risk status for one or more reasons. Thus, electronic fetal heart rate monitoring during transport would be beneficial if it can be accomplished despite the adverse conditions of turbulent flight and patient positioning. Monitoring of the fetus during transport is currently accomplished most frequently by intermittent Doppler auscultation. A prospective study was designed to examine the usefulness of electronic fetal heart rate monitoring during fixed-wing transport of obstetric emergencies. A second problem addressed by this study is the effect of altitude on fetal oxygenation.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Good Samaritan Medical Center.
Received for publication October 28, 1986; revised March 16, 1987; accepted April 27, 1987.
Reprint requests: John P. Elliott, M.D., c/o Division of Maternal-Fetal Medicine, Good Samaritan Medical Center, 1111 E. McDowell Rd., Phoenix, AZ 85006.

Material and methods

All patients included in this study were transported by Air Evac Air Transport Service operated by SamCor based in Phoenix, Arizona. The study population includes all fixed-wing maternal transports from October 13, 1985, to December 31, 1985. Three types of aircraft were used to transport patients: a twin-engine Cessna 414, a twin prop-jet 441 Conquest, and a twin prop-jet Gulfstream Turbo Commander 900. These aircraft were all pressurized and have been specially equipped for transporting pregnant patients. A Litton fetal heart rate monitor, model 550A (Litton Datamedix, Sharon, Massachusetts), was used during these transports, with an external Doppler ultrasonographic device for recording fetal heart tones and a tokodynamometer to detect uterine contractions. Five full-time and two part-time perinatal transport nurses accompanied all flights. All transport nurses have several years of obstetric experience in maternal-fetal intensive care. These nurses operated with protocol orders for medications and under the supervision of perinatologists in Phoenix. All patients were transported in left lateral position. The external monitors were applied and an attempt was made to monitor each transport. Twins were monitored intermittently because of the availability of only one Doppler recorder.

Monitor strips were evaluated by the authors with regard to quality of the tracing and the presence or absence of periodic fetal heart rate changes (accelerations, variable decelerations, and late decelerations). Maternal vital signs were monitored every 15 minutes during transport. Cruising altitude of the plane and cabin altitude were recorded.

Results

Fifty-seven emergency obstetric transports were performed by fixed-wing aircraft during the study period.

Table I. Indications for transport to level III tertiary hospital

Indication for transport	*Patients (No.)*
PROM or premature labor (<30 wk)	11
PROM or premature labor (>30 wk)	21
Preeclampsia	9
Eclampsia	2
Significant third-trimester hemorrhage	5
Pyelonephritis	2
CPD	2
Postdates (meconium)	2
Previous cesarean section in labor	2
Brain tumor	1
Total	57

PROM, Premature rupture of membranes; CPD, cephalopelvic disproportion.

Patients were transported from rural areas of Arizona, California, and New Mexico to one of three tertiary-level maternity hospitals in Phoenix. One-way mileage between Phoenix and these communities ranged from 184 to 598 statute miles. Air travel time ranged from 25 minutes to 80 minutes. Cruising altitude ranged from 9500 feet to 23,000 feet and cabin altitude maintained by pressurization was 1100 feet to 7000 feet. Of the 57 patients transported, 40 (70%) were monitored successfully with the electronic fetal heart rate monitor from a technical viewpoint. Seventeen patients (30%) were not monitored. Reasons for failure to monitor included: maternal obesity (three patients), no monitor available (four patients), no inverter (a device that allows a 120 v appliance to be operated from an airplane battery) available (five patients), and other (five patients). If a monitor was available and working, 40/48 (83%) of the patients were monitored successfully. The remaining 17 patients were monitored with an intermittent Doppler device. The indications for transport are summarized in Table I.

The 40 fetal monitor tracings were evaluated with respect to the quality of the data on the tracing concerning fetal heart rate and uterine contractions. Of the 40 strips available for review, 33 (83%) were considered "good" quality. "Good" quality was defined as a clear, readable tracing at least 50% of the time enroute. It was considered useful for evaluation of fetal heart rate and contraction frequency. Seven (17%) were rated as "poor" quality. "Poor" quality was defined as an unreadable tracing >50% of the time on the monitor. Accelerations of the fetal heart rate (>15 bpm) were recorded on 36 tracings. Contractions were present on 29 tracings. Three patients had mild variable decelerations during flight. Two of these patients had premature rupture of membranes and preterm labor and one patient had premature labor with intact membranes. There was no evidence of late decelerations on any of the tracings.

Comment

Regionalization of perinatal health care has been developing since the mid 1970s. Neonatal intensive care units developed at large urban hospitals evolved into high-risk centers where patients with obstetric and neonatal complications could be sent to use the centralized availability of specialists in high-risk obstetrics, newborn intensive care, ultrasonography, pulmonary medicine, anesthesia, cardiology, etc. Neonates whose mothers were transported ante partum to the tertiary center were compared with those transported after birth in several studies.[3-5] Conclusions supported by these data include increased survival, decreased short- and long-term morbidity, and decreased cost of hospitalization for the infants transported in utero and delivered at the tertiary center. The benefits of maternal transport with delivery of the infant at the tertiary center resulted in lower perinatal mortality and morbidity rates in other studies.[6-8]

Transportation of the high-risk pregnant patient has been accomplished by ambulance, helicopter,[8] and fixed-wing aircraft.[7] In sparsely populated regions, long transport distances may be necessary. To ensure safety, both mother and fetus must be monitored closely. Reasons for maternal transports frequently involve premature labor, and tocolytic drugs are often used to inhibit labor while moving the patient. Women with hypertension or significant vaginal bleeding may be at risk for fetal distress. The added potential hypoxia of transporting these patients at altitude in a fixed-wing aircraft must be evaluated.

In this study we were able to successfully monitor 70% of our patients on fixed-wing flights by electronic fetal heart rate monitoring. During nine transports a monitor was not available so, if these are eliminated, 83% of transported fetuses were monitored successfully. The remaining 17 patients were monitored by Doptone. Eighty-three percent (83%) of these tracings were judged to be adequate to evaluate uterine activity and fetal heart rate data. Only three patients demonstrated decelerations (all mild variables) and 36 out of 40 had accelerations (considered a reassuring sign of fetal well-being). Even the "poor" quality tracings were useful in that each of these had at least a 10-minute strip that often contained acceleration of fetal heart rate. Certain factors were considered important and affected the quality of the tracings: excessive fetal movement preventing adequate tracing, turbulence affecting the airplane and the monitor, maternal obesity, and improperly applied monitors (belts too loose or too tight). It was felt that the monitor was useful in ob-

taining adequate tracings of fetal heart rate during transport. These data could then be used for therapeutic interventions: administering oxygen, tocolytic drugs, change of maternal position, etc.

The effect of altitude on fetal oxygenation has been a question since transport by plane began. We attempted to evaluate this question clinically by observing the fetal heart rate tracings for signs of hypoxia. Murata et al.,[9] reported data from monkeys that suggests that as the fetus becomes more hypoxic and then acidotic, the first signs of hypoxia observed in the fetal heart rate are intermittent late decelerations followed by consistent late decelerations and finally a loss of acceleration that progressed to death as the Po_2 was lowered and acidosis became worse.

Although a crude observation, there were no late decelerations noted on any tracing in this study. This represents indirect evidence that fetal hypoxia did not occur during these flights. None of the patients had fetal distress at the referring hospital before transport. The use of pressurized cabins seems to have little or no effect on fetal oxygenation as measured clinically by fetal heart rate monitoring. This question would also concern pregnant flight nurses, airline attendants, or pilots. It appears that a flight with cabin pressures between 1100 feet and 7000 feet is safe for the fetus at least for short periods of flight up to 80 minutes.

In summary, the benefits of electronic fetal heart rate monitoring can be used during the relatively long time of fixed-wing transport to monitor the fetus more accurately than with intermittent Doptone. Sixty-six percent of all of the transported patients could be monitored adequately; however, there were some patients who could not be monitored for various reasons. These patients would be followed frequently with Doptone fetal heart rate. Electronic fetal heart rate monitoring can be a valuable tool during fixed-wing transport of the obstetric emergency patient and her fetus.

We thank the transport nurses who helped compile the data: Phyllis Borges, R.N., Denice Jarles, R.N., Pat Kroldart, R.N., Mary-Ellen Nelson, R.N., Dale Resnick, R.N., Susan Robertson, R.N., and Chris Rogers, R.N.

We dedicate this article to Pat Kroldart, R.N., who died during a transport in 1987.

REFERENCES

1. Modanlou HD, Dorchester WL, Thorosian A, Freeman RK. Antenatal versus neonatal transport to a regional perinatal center: a comparison between matched pairs. Obstet Gynecol 1979;53:725.
2. Merenstein GB, Pettett G, Woodall J, Hill JM. An analysis of air transport results in the sick newborn. II. Antenatal and neonatal referrals. Am J Obstet Gynecol 1977; 128:520.
3. Mirata T. Increased handicaps in transported very low birth–weight infants. Clin Res 1979;27:125A.
4. Harris TR, Isaman J, Giles HR. Improved neonatal survival through maternal transport. Obstet Gynecol 1978;52:294.
5. Harris BA, Wirtschafter DD, Huddleston JF, Perlis HW. In utero versus neonatal transportation of high-risk perinates: a comparison. Obstet Gynecol 1981;57:497.
6. Anderson CL, Aladjem S, Ayuste O, Caldwell C, Ismail M. An analysis of maternal transport within a suburban metropolitan region. Am J Obstet Gynecol 1981;140:499.
7. Souma MI. Maternal transport: behind the drama. Am J Obstet Gynecol 1979;134:904.
8. Elliott JP, O'Keeffe DF, Freeman RK. Helicopter transportation of patients with obstetric emergencies in an urban area. Am J Obstet Gynecol 1982;143:157.
9. Murata Y, Martin CB, Ikenoye T, et al. Fetal heart rate accelerations and late decelerations during the course of intrauterine death in chronically catheterized rhesus monkeys. Am J Obstet Gynecol 1982;144:218.

Congenital heart block and cardiac anomalies in the absence of maternal connective tissue disease

Lewis Shenker, M.D., Kathryn L. Reed, M.D., Caroline F. Anderson, R.D.M.S., Gerald R. Marx, M.D. Richard E. Sobonya, M.D., and Anna R. Graham, M.D.

Tucson, Arizona

The association of congenital heart block with maternal connective tissue disease and autoimmunity has been recently reported. Two cases of heart block were diagnosed in utero at 23 and 24 weeks' gestation. Both mothers had extensive workups for connective tissue disease that were negative. Two-dimensional and Doppler echocardiograpy were performed in utero and both infants had left atrial isomerism, atrioventricular canal defects, and severe hydrops in addition to heart block. Review of the recent literature identifies a subset of congenital heart block in which the mothers have no evidence for connective tissue disease, and the fetus or neonate has complex cardiac malformations and hydrops. The common features in these babies are atrioventricular canal defects and isomerism. No reports of congenital heart disease among the children of mothers with connective tissue disease fit this description. Our recent experience and literature review emphasize the previously known association between congenital heart block and congenital heart disease. This association is especially important in light of the poor prognosis for this group of fetuses and neonates. (Am J Obstet Gynecol 1987;157:248-53.)

Key words: Congenital heart block, atrioventricular canal defect, isomerism, polysplenia, asplenia, connective tissue disease

Congenital heart block can now be diagnosed prenatally[1] and its association with maternal connective tissue disease has been reported.[2, 3] We diagnosed congenital heart block in two fetuses whose mothers did not have clinical or laboratory evidence of connective tissue disease. Both fetuses had severe heart disease and hydrops. We reviewed the literature to determine whether this association was common. We found an association of congenital heart block with specific cardiac anomalies in the absence of maternal antibodies. Perinatal outcome in these cases was almost uniformly fatal.

From the Departments of Obstetrics and Gynecology, Pediatrics, and Pathology, University of Arizona Health Sciences Center.
Received for publication December 15, 1986; revised March 24, 1987; accepted April 28, 1987.
Reprint requests: Lewis Shenker, M.D., Department of Obstetrics and Gynecology, 1501 North Campbell, Tucson, AZ 85724.

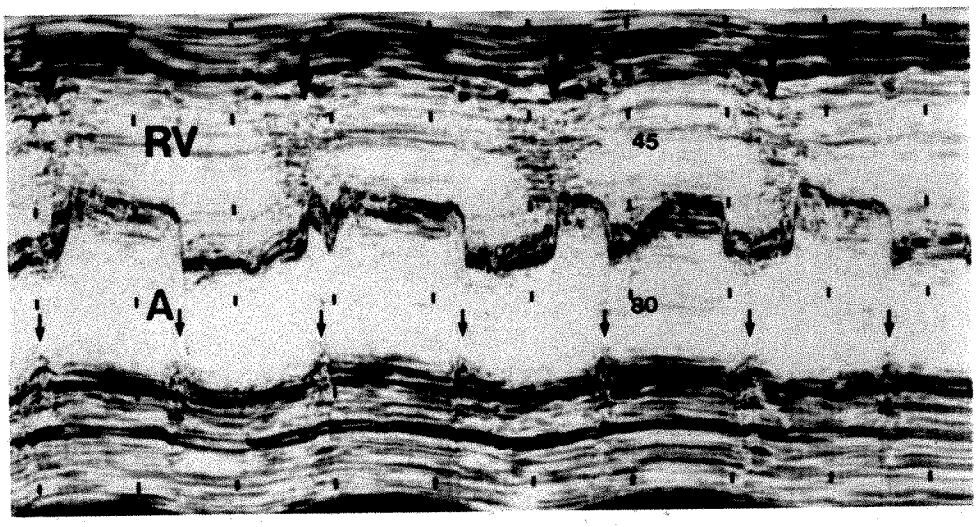


Fig. 1. M-mode echocardiogram of Case 1. Right ventricular *(RV)* contractions are labeled with *large arrows*. Atrial *(A)* contractions are labeled with *small arrows*. Leaflet motion is seen between atrium and ventricle. Vertical dots are 1 cm apart; paper speed is 2.5 cm/sec.

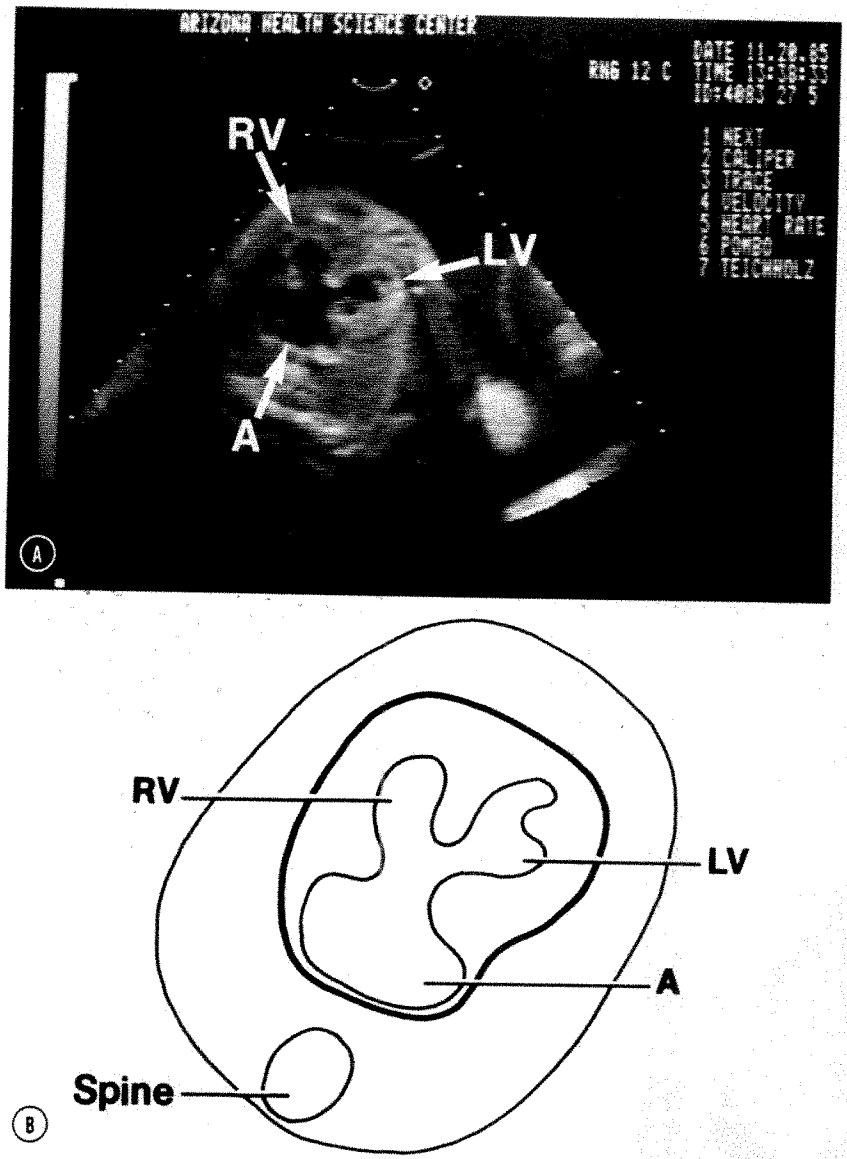


Fig. 2. Two-dimensional ultrasound and line drawing for Case 1. Ventricles are abnormally thick; the atrial septum is absent. *RV:* Right ventricle; *LV:* left ventricle; *A:* atrium.

Case reports

Case 1. Z. B., an 18-year-old black woman, gravida 2, para 1, had a fetal heart rate of 60 bpm reported at 23 weeks' gestation. The initial fetal cardiac ultrasound study, with two-dimensional, M-mode, and Doppler echocardiography, confirmed the diagnosis of complete heart block with an atrial rate of 80 bpm and a ventricular rate of 45 bpm (Fig. 1.) No ascites or pericardial and pleural effusions were present. Maternal anti-SSA, anti-SSB, and antinuclear antibodies were negative.

An ultrasound study at 26 weeks' gestation revealed persistent heart block with bilateral ventricular hypertrophy. The atria were dilated and no atrial septum could be identified. A common atrioventricular canal with a common atrium, single atrioventricular valve, and ventricular septal defect was diagnosed (Fig. 2). A small pericardial effusion was noted.

Ultrasound examinations were repeated at 1- to 2-week intervals. The outflow tracts appeared abnormal with a hypoplastic-appearing pulmonary artery and a dilated ascending aorta.

Genetic amniocentesis was performed; the karyotype was reported as 46,XX inv (4) (p14q21). Polyhydramnios and massive fetal edema with pleural effusions developed while the fetus was under observation. At 33 weeks' gestation, fetal movement had ceased and ultrasound examination confirmed the diagnosis of fetal death. Labor was induced and the patient was delivered of a stillborn breech infant weighing 1780 gm.

Postmortem examination revealed polysplenia with bilaterally left-sided bilobed lungs and left atrial iso-

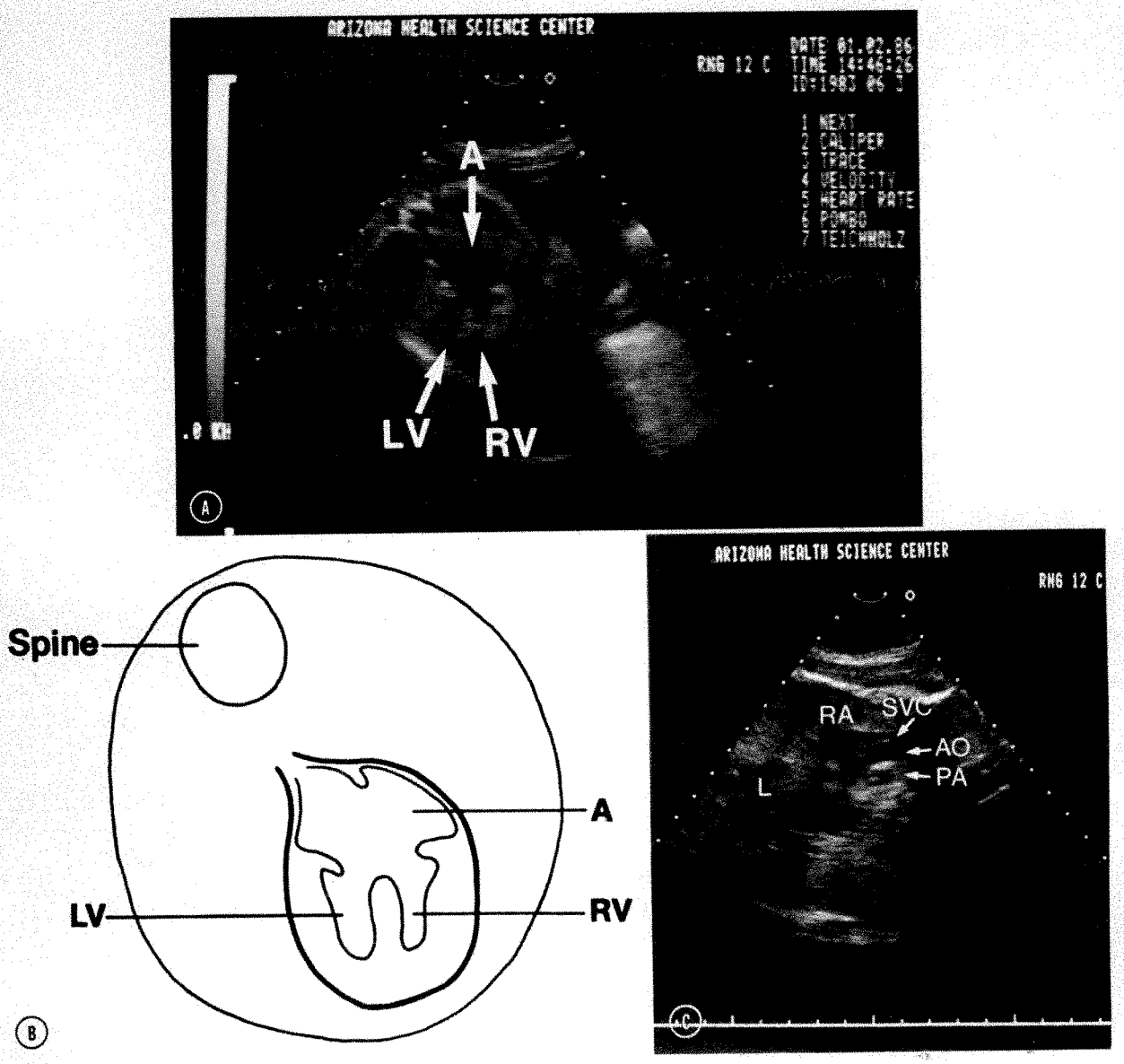


Fig. 3. Two-dimensional studies in Case 2. **A,** Two-dimensional ultrasound study. **B,** Line drawing. *A:* Atrium; *RV:* right ventricle; *LV:* left ventricle. **C,** Right side of atrium *(RA)* is seen with superior vena cava *(SVC)* entering; no inferior vena cava is seen. *AO:* Aorta; *PA:* pulmonary artery; *L:* liver.

merism. The suprarenal portion of the inferior vena cava was interrupted with azygous venous continuation into the superior vena cava. Bilateral superior venae cavae were identified. A common atrium, complete atrioventricular canal, and severe right ventricular outflow tract obstruction were present. Malalignment and inferior displacement of the parietal band with severe infundibular pulmonic stenosis and probable pulmonary atresia were found with a very hypoplastic main pulmonary artery. The aorta was dilated and overrode the interventricular septum. Cardiomegaly, pleural effusions (20 ml on each side), and pericardial effusion (5 ml) were confirmed.

Case 2. M. M., a 36-year-old woman, gravida 4, para 1, abortions 2, was referred at 22 to 23 weeks' gestation for fetal cardiac studies with a persistent fetal bradycardia (90 bpm). Anti-SSA, anti-SSB, and antinuclear antibodies were absent. Her first pregnancy was uncomplicated; her second pregnancy was terminated with the diagnosis of trisomy 21 at 16 weeks' gestation. Her third pregnancy resulted in a first-trimester spontaneous abortion. During the current pregnancy, chorionic villus sampling was performed at 8 weeks; the karyotype was 46, XX.

On fetal cardiac examination ventricular hypertrophy and an atrioventricular septal defect were diag-

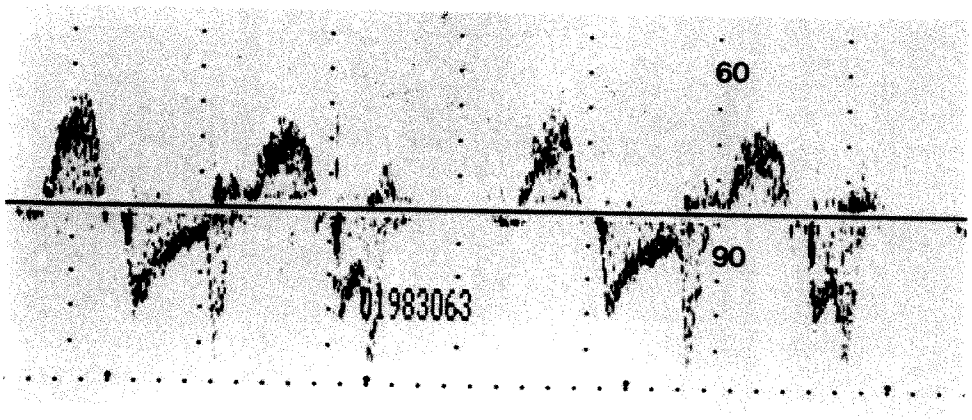


Fig. 4. Doppler echocardiogram of left ventricular inflow and outflow in Case 2. Ventricular (aortic outflow, above zero line) rate is 60 bpm; atrial (second peak of mitral inflow tracing, below zero line) rate is 90 bpm (3:2 block).

nosed (Fig. 3, *A* and *B*). The inferior vena cava was not identified (Fig. 3, *C*). Pleural effusions were present. On M-mode echocardiography, the atrial rate varied between 90 and 100 bpm; the ventricular rate was between 60 and 75 bpm. The rhythm was suggestive of a second-degree Mobitz type II block (Fig. 4).

By 32 weeks of pregnancy, polyhydramnios and fetal ascites had appeared. Cesarean section was performed at 33 weeks at the request of the parents, both of whom were physicians. The hydropic infant weighing 2600 gm had an Apgar score of 1 at 1 minute and 5 minutes. Despite initial attempts at resuscitation, ventilation of the lungs was unsuccessful and the infant died.

Postmortem examination revealed polysplenia, bilateral left-sidedness, and left atrial isomerism with an atrioventricular septal defect, bilateral superior venae cavae, and myocardial hypertrophy (Fig. 5). There was significant left ventricular outflow tract obstruction; the aortic anulus was narrowed, with a trileaflet aortic valve and poststenotic aortic dilation. A central liver, absence of the gallbladder, chronic stenosing cholangitis, and malrotation of the bowel were found. Ascitic fluid volume was 385 ml.

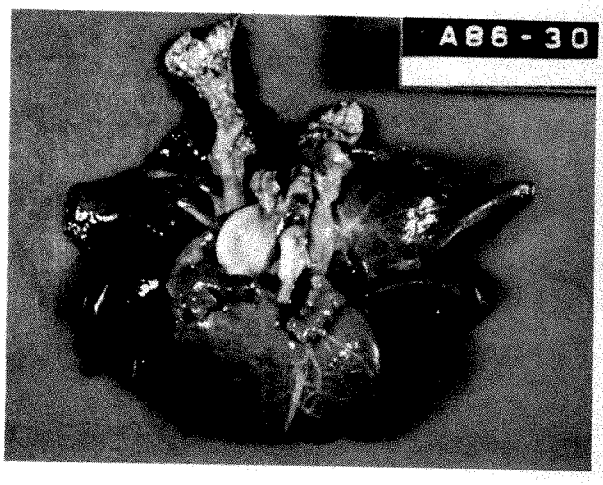


Fig. 5. Heart and lungs from Case 2. Cardiac enlargement, bilobed lungs, left atrial isomerism, and bilateral superior venae cavae are present. Note the disparity between the sizes of the great arteries.

Comment

Our findings in these two patients with congenital heart block without maternal connective tissue disease led us to a literature search to discover similar associations. Congenital heart block occurs in 1/20,000 neonates.[4] In many instances, maternal serologic test results positive for a variety of antibodies related to connective tissue disease have been described.[5] There were 60 cases with sufficient data to document the association between connective tissue disease in mothers of some babies with congenital heart block. In those patients, congenital heart block is accompanied by cardiac malformations in 23%. Twelve children had endocardial fibroelastosis and 14 had congenital heart disease. Six had a patent ductus arteriosus; two had dysplastic pulmonary valves; five had L-transposition. No complex cardiac abnormalities with isomerism were found.[3]

Review of the recent literature on congenital heart block discloses a group of patients in whom the diagnosis of congenital heart block was made either prenatally or in the immediate neonatal period and in whom all fetuses had complex congenital heart disease, usually accompanied by fetal hydrops. Twenty-six cases of congenital heart block accompanied by congenital heart disease diagnosed in the antepartum or immediate neonatal period have been reported since 1979 (Table I). The mothers of these children had no clinical or laboratory evidence of connective tissue disease. In each of these cases, the cardiac anomalies of atrioventricular septal defects and isomerism were present. Case 6 met all the criteria for left isomerism, but

Table I. Congenital heart block with congenital heart disease and isomerism

	Gestational age at diagnosis	*Atrial isomerism*	*Fetal hydrops*	*Mode of delivery*	*Outcome*
Okada[6]					
Case 1: No clinical data					
Garcia et al.[7]					
Case 2	12 days old	+			Died
Case 3	3 weeks old	+			Died
Case 4	2 months old	+			Died
Case 5	2 months old	+			Died
Crawford[8]					
Case 6	24 weeks' gestation	+	+	C/S	Died 1 mo
Case 7	32 weeks' gestation	+	+		Died 1 hr
Arnold et al.[9]					
Case 8	Birth	+	?		Died 6 wk
Kleinman et al.[10]					
Case 9	28 weeks' gestation	+	+		Died 10 min
Ih et al.[11]					
Case 10	2 months old	+	?		Died 2 min
Mehta and Sanchez[12]					
Case 11	Birth	+	+	C/S	Survived
Case 12	38 weeks' gestation	+	+	C/S	Died 1 day
Roguin et al.[13]					
Case 13	Birth	+	+		Died 12 days
Case 14	Birth	+	+		Died
Crawford et al.[14]					
Case 15	Second trimester	+	+	Abortion	
Case 16	Second trimester	+	+	Abortion	
Case 17	33 weeks' gestation	+	+		Stillborn
Case 18	38 weeks' gestation	+	+	C/S	Died 2 yr
Iversen et al.[15]					
Case 19: No clinical data					
Copel and Kleinman[1]					
Case 20	29 weeks' gestation	+	+		Died 36 wk
Stewart et al.[16]					
Case 21	21 weeks' gestation	+	+	Abortion	
Case 22	29 weeks' gestation	+	+		Stillborn
Silverman et al.[17]					
Case 23	29 weeks' gestation	+	+		Died 1 day
Copel and Kleinman[1]					
Case 24	27 weeks' gestation	+	+		Died 1 day
Shenker et al. (present article)					
Case 25	23 weeks' gestation	+	+		Stillborn
Case 26	24 weeks' gestation	+	+	C/S	Died 2 hr

C/S = Cesarean section.

polysplenia was not described. Case 19 had only a "large ventricular septum defect" on postmortem examination. Twenty-one fetuses and neonates had ultrasound, cardiac catheterization studies, angiography, and/or autopsy findings diagnostic of left isomerism with polysplenia. Four cases (Cases 21 to 24) had right isomerism and asplenia.

Twenty percent of patients with structural heart disease and isomerism have heart block.[13] All fetuses or neonates with congenital heart block diagnosed prenatally or shortly after birth who had structural cardiac anomalies uniformly had predictable major and complex lesions of the heart as well as either left or right isomerism. To date, no reported mothers with laboratory or clinical evidence of connective tissue disease have been delivered of babies with isomerism. These facts are consistent with different pathologic mechanisms for the conduction abnormality and the structural abnormalities, depending on the presence or absence of abnormal maternal antibodies typical of connective tissue disease.

The pathologic basis for complete heart block in patients with left isomerism has been described by Ih et al.[11] There is duplication of the atrioventricular node; the separate nodes are connected by a sling of conduction tissue. In three patients with congenital heart block, an interruption of the connection of the sling to the ventricular bundles was seen. Garcia et al.[7] stated that the sling is an "anatomical substrate for the development of heart block" in patients with left isomerism. They described two patients who had normal sinus rhythm converting to heart block over a period of years.

The development of heart failure in utero manifested by fetal hydrops appears to be an inevitable com-

plication of the combination of atrial isomerism, complex cardiac malformations, and heart block. Hydrops was reported in almost every case with clinical information available. Only one surviving patient has been described. When the diagnosis was made prenatally or at birth, there were 17 neonatal deaths, three stillbirths, and three induced abortions.

The patients reviewed and reported on manifested the most severe expression of isomerism. When isomerism is accompanied by heart block, atrioventricular septal defects, and hydrops, the affected fetus is almost uniformly unsalvageable. Despite the advances in pacemaker therapy of congenital heart block uncomplicated by anatomic abnormalities, the very high perinatal mortality of the group of patients described is discouraging. Our experience and the literature review suggest that cesarean section for a fetal indication is not justifiable.

Once the diagnosis of heart block has been confirmed by appropriate ultrasound studies and other causes of slow fetal heart rates such as persistent nonconducted atrial premature beats have been ruled out, the fetal heart must be carefully examined. Special attention should be directed to determine whether an atrioventricular canal defect, inferior vena cava, and abnormal pulmonary artery or aorta are present. Maternal antibody studies, such as antinuclear antibodies, anti–deoxyribonucleic acid antibodies, anti-Ro (anti-SSA) antibodies, and anti-La (anti-SSB) antibodies should be done, and a history and physical examination of the mother, particularly for the presence of connective tissue disease, should be performed. In the presence of fetal cardiac anomalies, a genetic amniocentesis is recommended, especially with the increased association of Down syndrome with atrioventricular canal defect. If hydrops is not present, expectant management is appropriate, and vaginal delivery is safe. In the presence of hydrops with a normal karyotype and without major abnormalities, delivery by cesarean section when lung maturity has been confirmed, or sooner if fetal surveillance indicates deterioration in fetal condition, is desirable. The hydropic fetus without lethal cardiac anomalies should not be subjected to labor. If isomerism, atrioventricular septal defect, and hydrops are diagnosed, interruption of pregnancy or the avoidance of cesarean section for fetal indications is recommended. It is important to include the consultation of pediatric cardiologists and neonatologists to individualize intrauterine and neonatal management.

REFERENCES

1. Copel JA, Kleinman CS. The impact of fetal echocardiography on perinatal outcome. Ultrasound Med Biol 1986;12:327-35.
2. McCue CM, Mantakas ME, Tingelstad JB, Ruddy S. Congenital heart block in newborns of mothers with connective tissue disease. Circulation 1977;56:82-90.
3. Litsey SE, Noonan JA, O'Connor WN, Cottrill CM, Mitchell B. Maternal connective tissue disease and congenital heart block. N Engl J Med 1985;312:98-100.
4. Michaelsson M, Engle MA. Congenital complete heart block. An international study of the natural history. Cardiovasc Clin 1972;4:86-101.
5. Scott JS, Maddison PJ, Taylor PV, Esscher E, Scott O, Skinner RP. Connective-tissue disease, antibodies to ribonucleoprotein, and congenital heart block. N Engl J Med 1983;309:209-12.
6. Okada R. Congenital heart disease. Histology of the conduction system in partial transposition with common atrioventricular orifice associated with polysplenia and congenital AV block. Nippon Rinsho 1979;37:3564-7.
7. Garcia OL, Mehta AV, Pickoff AS, et al. Left isomerism and complete atrioventricular block: a report of six cases. Am J Cardiol 1981;48:1103-7.
8. Crawford CS. Antenatal diagnosis of fetal cardiac abnormalities. Ann Clin Lab Sci 1982;12:99-105.
9. Arnold GL, Bixler D, Girod D. Probable autosomal recessive inheritance of polysplenia, situs inversus and cardiac defects in an Amish family. Am J Med Genet 1983;16:35-42.
10. Kleinman CS, Donnerstein RL, Jaffe CC, et al. Fetal echocardiography. A tool for evaluation of in utero cardiac arrhythmias and monitoring of in utero therapy: analysis of 71 patients. Am J Cardiol 1982;51:237-43.
11. Ih S, Fukada K, Okada R, Saitoh S. The location and course of the atrioventricular conduction system in common atrioventricular orifice and in its related anomalies with transposition of the great arteries—a histopathological study of six cases. Jpn Circ J 1983;47:1262-73.
12. Mehta AV, Sanchez GR. Left isomerism (polysplenia syndrome) and complete atrioventricular block. Am J Cardiol 1983;52:429.
13. Roguin N, Pelled B, Freundlich M, Yahalom M, Riss E. Atrioventricular block in situs ambiguus and left isomerism (polysplenia syndrome). PACE 1984;7:18-22.
14. Crawford D, Chapman M, Allan L. The assessment of persistent bradycardia in prenatal life. Br J Obstet Gynaecol 1985;92:941-4.
15. Iversen O, Lossius P, Lovset T, Finne PH. Complete fetal heart block diagnosed by ultrasound. Acta Obstet Gynecol Scand 1985;64:533-5.
16. Stewart PA, Tonge HM, Wladimiroff JW. Arrhythmia and structural abnormalities of the fetal heart. Br Heart J 1983;50:550-4.
17. Silverman NH, Enderlein MA, Stanger P, Teitel DF, Heymann MA, Golbus MS. Recognition of fetal arrhythmias by echocardiography. JCU 1985;13:255-63.

Factors affecting psychological adjustment to a fetal death

Melody A. Graham, M.A., Suzanne C. Thompson, Ph.D., Mary Estrada, R.N., and Margaret Lynn Yonekura, M.D.

Los Angeles, California

The loss of a child in utero can be a tragic experience. The purpose of the present study was to examine some patient characteristics that may predict which women are likely to have problems in adjusting to the loss and to examine the effectiveness of interventions by care providers in facilitating emotional recovery. Twenty-eight women who were no more than 4 weeks post partum from a fetal death were interviewed while waiting to see their physicians at the fetal demise clinic at Los Angeles County Women's Hospital. It was found that women were less depressed after the loss if they already had children, if they did not blame themselves for the death, and if they received a picture of the infant or were allowed to see the infant. Women benefited from sympathy from the medical personnel and being kept informed of problems as they developed. (AM J OBSTET GYNECOL 1987;157:254-7.)

Key words: Coping, fetal death, blame, psychological adjustment

The loss of a child in utero is similar to the death of any loved one and is often associated with significant long-term psychological consequences.[1, 2] Unfortunately, patients often must carry the burden of the death alone because medical personnel, friends, and family members may not realize the extent of attachment that a mother has for her unborn infant and thus they underestimate the grief and sorrow that she feels.[3]

Not a lot is known about what affects positive psychological adjustment to this type of loss. The purpose of the present study was to examine some patient characteristics that may predict which women are likely to have problems in adjusting to the loss and to examine the effectiveness of interventions by care providers in facilitating emotional recovery.

Previous research using a cognitive adaptation model of coping has found that individual responses to a traumatic event depend on the meaning of the loss to the person experiencing it.[4] The meaning of a fetal death is likely to be affected by three patient characteristics: how much the woman wanted the child, whether she has other children, and whom she blames for the loss. The assignment of blame can be particularly important because these judgments are associated with feelings of being treated unfairly, the perception that a similar event can be avoided in the future, and distressing ruminations about the event. We measured these three patient characteristics to see if they predicted how well the women were coping with the loss.

The effects of two interventions on the part of medical care providers were also examined: providing the mother with a picture of the infant or an opportunity to see the infant and informing the mother about the cause of death. The recent literature suggests that it is helpful for parents to see their dead infant (despite deformities), hold the infant, name it, and bury it.[5, 6] Some hospitals have begun to take pictures of the child and give death certificates and mementos of the child to the parents to help establish the child's identity. However, the effectiveness of these procedures has not been established in a controlled study. Therefore, one goal of this study was to see if mothers who received pictures of the child or saw the infant would find it easier to deal with the loss.

If was also expected that knowledge of the cause of the infant's death would facilitate the recovery process. Research on other types of losses has found that it is particularly difficult for people to deal with a misfortune when they do not understand why it happened to them.[4] Also, guilt is the most common postmiscarriage emotion.[7] Knowing the cause of death may help alleviate some of this guilt.

Finally, the study examined women's perceptions of the ways in which medical personnel were supportive and helpful to them in dealing with the loss and ways in which the medical personnel were not helpful. It was hoped that the women's observations would provide additional information about ways in which care providers could aid in the recovery process.

Material and method

Twenty-eight women who were no more than 4 weeks post partum from a fetal death or perinatal death were interviewed while waiting to see the physician at the fetal demise clinic at Los Angeles County Women's Hospital, University of Southern California

From Claremont Graduate School, Pomona College, and University of Southern California School of Medicine.
Received for publication October 10, 1986; revised February 6, 1987; accepted March 12, 1987.
Reprint requests: Melody A. Graham, Psychology Department, Claremont Graduate School, Claremont, CA 91711.

Medical Center. The fetal demise clinic was established to ensure more consistent antepartum and postpartum medical care for patients who have experienced a fetal death. This clinic allows women to receive postpartum checkups and antepartum care in a setting away from women who have had a successful pregnancy. The clinic was designed to not only give consistent medical care to these patients but also attend to the psychological and emotional needs of the patient. Physicians are directed to give special attention to the patient's emotional needs, and patients who are particularly disturbed by the incident are referred to a social worker. Patients are encouraged to see, touch, and hold their infants if they feel comfortable doing so. Pictures are also taken of the child and offered to the parents. However, because the hospital is often overcrowded, these procedures may not always be completed. Therefore not all families are offered a chance to hold their child, and pictures are not always taken. All patients are encouraged to have an autopsy performed. However, a large number of patients refuse to have this procedure completed. The goal of the clinic is to have a supportive facility in which patients can be treated. However, because of overcrowding and understaffing the clinic does not always meet these high standards.

The average age of the respondents was 30 years, ranging from 15 to 39 years (mode 21). Ninety-six percent of the sample were Hispanic and spoke little or no English. On average, the respondents had been in the United States for 7 years. The sample was also very religious, with 92% of the respondents reporting that they considered themselves religious (56% considered themselves very religious).

The 30-minute interview was conducted by a nurse employed by the hospital. This nurse did not normally work in the fetal demise clinic. At the end of the interview the depression subscale of the Symptom Check List (SCL-90) was administered to each patient as a measure of coping. The SCL-90 is a multidimensional self-report symptom inventory oriented toward the measurement of psychological symptomatic distress. Information was also collected from the patient's medical chart. Family members were not allowed in the room during the interviews to ensure that the patient felt free to answer all questions. Interviews were conducted in the respondent's native language and tape recorded. Pearson correlation coefficients (r) were used to analyze the data.

Results

The fetus was carried an average of 36 weeks and weighed an average of 2373 gm. More than half the sample had pregnancy complications such as breech births and diabetes. This was the first pregnancy for 32% of the women, 36% were taking medications during their pregnancy, and 19% had received no prenatal care.

As a measure of attachment, women were asked about their feelings about being pregnant. Fifty-four percent of the women reported being very happy about being pregnant, 12% were unhappy about being pregnant, and the rest (34%) felt somewhat happy. However, degree of happiness about being pregnant was not related to depression for this sample.

Family situation. Although only 42% of the sample were married, 58% lived with the father of the infant. Married respondents were significantly less depressed than those who were single ($r = 0.39$; $p < 0.05$). Fifty-seven percent of the women had children already and 27% had more than two children. The number of children the respondent already had was also significantly related to depression; the more children a respondent had, the less depressed she was ($r = -0.43$; $p < 0.05$).

Blame. The most common attribution of blame for the death of the child was to God. Thirty-one percent of the people who attributed the death to God reported feeling that way "all of the time." Those who attributed the death to God were also significantly less depressed ($r = -0.33$; $p < 0.05$). Blaming oneself for the death was more common than blaming another person, but still only one fourth of the respondents reported feeling that something they did caused the infant to die. However, those who blamed themselves were significantly more depressed ($r = 0.34$; $p < 0.05$). Those who did blame themselves had difficulty articulating why they felt responsible. One woman stated, "I don't remember doing anything, but I think that way (blame myself) anyway." Women who were taking medication during the pregnancy were more likely to blame themselves for the death ($r = 0.36$; $p < 0.05$).

Nineteen percent of the women blamed some member of the medical profession for their child's death. Blaming the medical personnel for the death did seem to cause feelings of being cheated by the death, but was not significantly related to depression. All of the women who blamed the medical personnel felt cheated by the death and also felt that the death could have been avoided (for example, "The clinic doctor was irresponsible, in the morning there was a heartbeat by evening there wasn't a heartbeat, they should have done something." "The clinic doctors were trainees and didn't know what they were doing, I felt like a guinea pig . . . they can't even take blood right." "The doctor at the clinic was not the right doctor to see women with first pregnancies, I was supposed to see someone else.").

As can be seen by these remarks, the blame is not always warranted or justified (for example, all physicians at the clinic mentioned do the same thing) but demonstrates the need to find someone who is responsible for the death. As stated before, other-blame was

associated with strong feelings of being cheated and the sense that the death was unfair.

A common theme in the women's remarks was that the death was not fair because they had been told everything was going to be all right (for example, "Up till the end my baby was fine, it is not fair." "When I was experiencing the labor pains everyone was saying everything was fine with my baby, *then* they told me the baby was not all right."). Thus it appears that being told everything is going to be "ok" when it is not leads to stronger feelings that the death was unfair.

Cause of death. The leading cause of death was cord accidents (30%); 12% died of fetal anomalies. The cause of death was unknown in 27% of the cases. Even though the cause of death was known in most cases, only 42% of the respondents reported knowing why the infant died. This may be because respondents were interviewed before seeing the physician. Contrary to prediction, knowing the cause of death did not have an effect on depression. However, those who did know the cause of death were more likely to rate the physician as being helpful ($r = 0.32$; $p < 0.05$).

Pictures. Forty percent of the respondents received pictures of the infant. Those who received pictures were significantly less depressed than those who did not ($r = 0.35$; $p < 0.05$). Eighty-four percent were allowed to hold or see the infant. These respondents were also significantly less depressed ($r = 0.38$; $p < 0.05$). Because the criteria for who receives a picture or who sees the infant are somewhat arbitrary, differences among the groups were checked. Those who received pictures or saw the infant did not differ significantly from those who did not receive pictures on marital status, number of children, age of infant, or weight of infant at birth. However, women who received pictures of the infant were less likely to have had complications in birth ($r = -0.36$; $p < 0.05$).

Seeing pictures of the infant was also significantly related to variables other than depression. Women who saw pictures were less likely to blame themselves for the death ($r = 0.39$; $p < 0.05$) and were less likely to blame another person ($r = 0.39$; $p < 0.05$). Thus it appears that receiving pictures does have the positive effect that has been suggested in the literature.

However, one explanation for the finding that those who received pictures were less depressed is that women who were more emotionally devastated by the loss refused to accept pictures of their infants if they were offered. No record was kept of refusals, but we did have a measure of whether each woman had wanted to receive a picture. No one who received a picture mentioned that she did not want to receive the picture, but some women who did not receive pictures stated that they did not want them. Presumably, some of these women may have refused pictures that were offered to them. However, women who did not want and did not receive pictures were less depressed than those who wanted but did not receive them, so it is unlikely that the higher depression scores of those who did not receive pictures can be attributed to women who were more upset refusing the pictures.

Helpful and unhelpful support. Forty-six percent of the respondents rated the medical personnel as helpful almost always or most of the time. The majority of these respondents reported that what they found helpful was that the physician and nurses gave them sympathy. When asked for specific examples of this, respondents reported things like, "The nurses stayed with me and talked to me" or "The doctor gave me sympathy and tried to console me." Thirty-five percent found the medical personnel unhelpful. However, none of these women reported anything in particular as unhelpful. This may be because the interviews were conducted in the hospital by a nurse, so respondents may have been unwilling to mention specific criticisms of the medical staff. However, what the women who were less satisfied did report was that their physicians were helpful in only very limited ways (for example, by only providing the name of a cemetery). They did not feel that they received any emotional support from the medical personnel.

Comment

This study found that women who lost a child in utero were less depressed if they already had children. Presumably, these women knew that they could deliver a healthy child, so the death was less likely to arouse a sense of failure or responsibility for failed reproductive performance. This suggests that women who lose their first child in utero may be particularly likely to benefit from information that a healthy delivery is still possible for them.

The way that women attributed blame for the death was also related to psychological adjustment. Those who blamed themselves for the loss were more depressed than those who did not. Previous research on coping with a loss had found that self-blame is adaptive if it engenders feelings of control and the perception that one can act to avoid a similar misfortune in the future.[8] Evidently, women who blamed themselves in this study did not see their role in the loss as something that they could change and therefore avoid in the future. Because self-blame for this type of loss does not appear to be adaptive, it might be useful, where appropriate, for physicians to reassure women that they are not responsible for the loss.

The third patient characteristic measured, attachment to the child or how much the woman wanted the child, was not related to depression. This is surprising because one would expect that women who were more

emotionally attached to the unborn child would be more devastated by the loss. There are two explanations for this finding. First, asking the mother how she felt about the pregnancy is not an adequate measure of attachment. Further research with other ways of assessing attachment could test this explanation. Second, perhaps by the late stages of a pregnancy almost all mothers are attached to the unborn child, regardless of whether the pregnancy was desired. Given that there is a high level of attachment by most mothers, other factors such as who is blamed for the death or whether the mother has other children are more predictive of differences in coping with the loss.

Contrary to what was expected, knowing the cause of death was not related to how depressed a woman was. It appears that understanding the cause of one's misfortune is not as important for this type of loss as it has been found to be for other negative experiences. However, this may be a culturally based finding. The participants in this study had been in the United States for an average of only 7 years. A different cultural group may find it more important to understand the cause of death.

Seeing the infant or receiving a picture of the infant appeared to be quite helpful for the respondents. This is a fairly easy intervention that can be implemented in hospitals and may make recovery from the loss easier. Not only did those who received pictures feel less depressed, they also did not blame themselves or another person as much. Seeing a picture or seeing the infant allows the parents to mourn a real child and makes the event more real.

The women's observations on helpful and unhelpful behaviors of medical personnel suggest two things. One, women in these circumstances seem to appreciate and benefit from shows of sympathy and understanding from medical care providers. Physicians who can provide emotional support during interactions with the patient may help the recovery process. Two, women find it especially stressful to lose a child shortly after they had been reassured that there were no problems. In some cases, when complications are not evident immediately, it may be impossible to avoid giving misleading information. However, it appears that whenever possible, women should be kept informed of problems that are developing.

One limitation of the study should be noted. The women's adjustment to the loss was examined 4 weeks post partum, a time that is relatively early in the grieving process. There was a wide range of depression scores suggesting that some women were coping better at this time than were others, and one would expect that those who were handling the grief well at this stage would continue to be the more effective copers. However, the suggestions drawn from this study are most applicable to those patients in the acute stages of grief.

The loss of a child in utero is a tragic experience for most women. This study points out a number of ways that medical care providers can ease the recovery process for women faced with such a loss.

REFERENCES

1. Carr D, Knupp SE. Grief and perinatal loss: a community hospital approach to support. J Obstet Gynecol Neonatal Nurs 1985;14(2):130-9.
2. Speck WT, Kennell JH. Management of perinatal death. Pediatr Rev 1980;2:59-62.
3. Leppert PC, Pahlka BS. Grieving characteristics after spontaneous abortion: a management approach. Obstet Gynecol 1984;1:119-22.
4. Janoff-Bulman R, Frieze I. A theoretical perspective for understanding reactions to victimization. J Soc Issues 1983;39:1-17.
5. Kirk EP. Psychological effects and management of perinatal loss. Am J Gynecol 1984;149:46-51.
6. LaFarla JJ, Good RS. Helping patients cope with pregnancy loss. Contemp OB/GYN 1985;26:107-15.
7. Bourne S, Lewis E. Pregnancy after stillbirth or neonatal death. Lancet 1984;7:31-3.
8. Janoff-Bulman R. Characteriological versus behavioral self-blame: inquiries into depression and rape. J Pers Soc Psychol 1979;31:1798-1809.

Erb's palsy and fetal bruising after external cephalic version: Case report

Boris M. Petrikovsky, M.D., Hema N. DeSilva, M.D., and Fred D. Fumia, M.D.
Farmington and Hartford, Connecticut

An unusual fetal complication, Erb's palsy and fetal bruising, after successful external cephalic version is reported. Importance of fetal weight estimation prior to the attempts to perform a version is stressed. (AM J OBSTET GYNECOL 1987;157:258.)

Key words: External cephalic version, fetal trauma, Erb's palsy.

External cephalic version is known to be associated with obstetric (premature labor, bleeding, etc.) and fetal (fractured bones, spinal cord injuries, prolonged bradycardia requiring emergency cesarean section, umbilical cord prolapse, meconium aspiration) complications. Antepartum fetal death is a rare but grave accident. In a large series of Bradley-Watson,[1] five of the eight deaths occurred after successful version and three after a failed procedure. To our knowledge, however, no case of antenatal peripheral nerve injury has been reported. We present such a case.

Case report

A 33-year-old woman, para 1-0-1-1, was admitted at 41 weeks' gestation with breech presentation for an external cephalic version. The patient had an uncomplicated pregnancy. Ultrasound examination confirmed a term gestation and breech presentation. The placenta was located posteriorly. Version was successfully performed under sonographic guidance with the use of a tocolytic agent without difficulty. A nonstress test done after the version was reactive. Five hours after version, the patient went into labor and was delivered of a 4.11 kg infant by vacuum extraction. The vaccum extractor was applied when the head was directly OA at +3 station. The anterior left shoulder was delivered easily, and the infant cried spontaneously, receiving Apgar scores of 8 and 9 at 1 and 5 minutes, respectively. Physical examination at delivery revealed Erb's palsy on the left side as well as bruising of the left shoulder and adjacent areas of the anterior and posterior chest wall. Radiologic evaluation did not reveal clavicular or humeral fractures or subluxation of the left shoulder.

During the hospital stay the infant showed improvement in movement of the left upper extremity with some limitation of abduction at the shoulder. Progressive improvement has been seen on follow-up at 2 weeks. Only mild residual weakness from Erb's palsy was still present in the fourth week.

Comment

The presence of visible bruises at the time of delivery, in view of the easy delivery of the shoulder girdle, can be ascribed to external cephalic version. We think that fetal weight should be estimated prior to the procedure and version not undertaken in the case of a large fetus. The fact that external cephalic version is "easy" and successful does not guarantee its safety for the fetus. Immediate effects of external cephalic version on the fetus are unknown because of the prolonged period of time between the procedure and delivery. In Ellis' series[2] of 314 external cephalic versions only four infants were delivered within 7 days of the attempted procedure.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, the University of Connecticut Health Center, Farmington, and the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, and the Division of Neonatology, Department of Pediatrics, St. Francis Hospital and Medical Center, Hartford.
Received for publication November 21, 1986; revised February 7, 1987; accepted March 3, 1987.
Reprint requests: Boris M. Petrikovsky, M.D., The University of Connecticut Health Center, Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, 263 Farmington Ave., Farmington, CT 06032.

REFERENCES

1. Bradley-Watson RJ. The decreasing value of external cephalic version in modern obstetric practice. AM J OBSTET GYNECOL 1975;123:237.
2. Ellis R. External cephalic version under anesthesia. J Obstet Gynaecol Br Commonw 1968;75:865.

A reusable plastic training model for teaching Papanicolaou smear technique

Charles R. B. Beckmann, M.D., M.H.P.E., Daniel L. Clarke-Pearson, M.D., and Ray Evenhouse

Chicago, Illinois

A reusable plastic Papanicolaou smear training model was developed to teach the techniques of the Papanicolaou smear. The model design allows immediate feedback and opportunity for skills practice, yet it is efficient and cost effective. Its use is recommended for instruction in the techniques of the Papanicolaou smear. (Am J Obstet Gynecol 1987;157:259-60.)

Key words: Papanicolaou smear, medical education, training model

Some physicians avoid performing Papanicolaou smears as part of a pelvic examination because they are uncomfortable with the techniques. Others perform the examinations but because of poor technique produce a Papanicolaou smear that is inadequate for cytopathologic evaluation. It is reasonable to assume that the difficulties physicians experience are related in part to inadequate training. Training in the Papanicolaou smear technique usually accompanies instruction in the pelvic examination, which has improved greatly with the adoption by most medical schools of the gynecologic teaching associate model.[1] Performance of the Papanicolaou smear, however, is different from the remainder of the pelvic examinations in that it is strictly a psychomotor skill so that the benefits of the gynecologic teaching associate training system may not carry over to instruction in the Papanicolaou smear. Further, many educators and gynecologic teaching associates have reservations about gynecologic teaching associates having multiple Papanicolaou smears.

To provide improved instruction in the Papanicolaou smear technique while remaining within the gynecologic teaching associate teaching model, we developed a plastic reusable Papanicolaou smear training model. It is composed of three parts: a clear plastic square support base, a clear plastic tube to simulate the vagina,

From the Department of Obstetrics and Gynecology, University of Illinois College of Medicine at Chicago, and Biocommunications Arts, University of Illinois at Chicago.

Received for publication August 7, 1986; revised January 8, 1987; accepted January 22, 1987.

Reprint requests: C. R. B. Beckmann, M.D., M.H.P.E., Department of Obstetrics and Gynecology, University of Illinois Medical Center, 840 S. Wood St., Chicago, IL 60612.

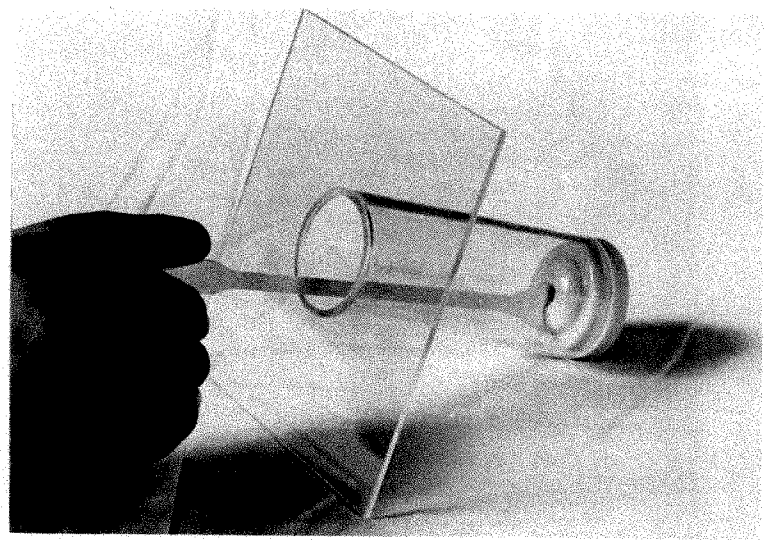


Fig. 1. The reusable plastic Papanicolaou smear training model.

and a molded, flesh-colored, soft rubber artificial cervix. The latter is molded after the endocervical end of a Papanicolaou smear spatula so as to facilitate practice. The parts are interchangeable between the training models, which may be disassembled for easy storage and cleaning. The design of the training model allows both the instructor and the student to observe the student's practice from the side-on vantage point and from an end-on vantage point (see Fig. 1). Of the two vantage points, the former is impossible in an actual patient but is of considerable value in guiding the student toward acceptable technique.

After viewing a videotape demonstrating the technique of the Papanicolaou smear with the plastic training model, students practice the techniques of the Papanicolaou smear, including correct maneuvers with spatula and cotton-tipped applicator, in obtaining the sample and applying it to a slide. Clear lubricant is applied to the end of the artificial cervix so that there is a "sample" to practice spreading on slides. Each student is allowed to practice the Papanicolaou smear technique under faculty supervision until his technique is satisfactory.

Use of the plastic Papanicolaou smear training model in this manner allows emphasis of the essential principles of the Papanicolaou smear technique: (1) proper sampling of the endocervix with cotton applicator, brush, or aspirator; (2) proper sampling of the exocervix with the wooden or plastic Papanicolaou smear spatula; (3) proper spreading of cytopathologic material on the glass slides in a manner that avoids clumping of the mucus on the slide; (4) rapid fixation of the slides to avoid air-drying artifact of the specimen. These essential principles may be emphasized by immediate feedback based on direct observation of the student's performance and by the opportunity for practice until the student has mastered the techniques. Such feedback and the opportunity to practice are key to successful skills instruction.[2]

The plastic Papanicolaou smear training model has been used for 1 year in the instruction of second- and third-year medical students and resident physicians. Both groups of learners, disparate in their experience, uniformly expressed the opinion that instruction on the technique of the Papanicolaou smear by use of the plastic training model was efficient and effective. Studies are now underway to ascertain if there is an improvement in the quality of Papanicolaou smears received. On the basis of our experience with this training model, we are confident in recommending it for instruction in the techniques of the Papanicolaou smear.

REFERENCES

1. Beckmann CRB, Spellacy WN, Yonke A, Barzansky B. Initial instruction in the pelvic examination in the United States and Canada, 1983. AM J OBSTET GYNECOL 1985; 151:58.
2. Foley R, Smilansky J. Teaching techniques: a handbook for health professionals. New York: McGraw-Hill, 1960.

Multiple primary gynecologic neoplasms

Peter G. Rose, M.D., Edward E. Herterick, B.S., John G. Boutselis, M.D., Melvin Moeshberger, Ph.D., and Larry Sachs, Ph.D.
Columbus, Ohio

Some patients may be predisposed to the development of more than one gynecologic neoplasm. We evaluated 130 cases of synchronous or metachronous tumors among 5967 patients followed up by The Ohio State University Gynecologic Tumor Registry for the past 44 years from 1939 to 1983. Based on primary tumor site and invasive behavior, expected incidences for a specific second malignancy were calculated by the person-years method. A second malignancy of the lower genital tract occurred in patients with cervical, vulvar, and vaginal cancers, 1.6%, 4.3%, and 9.6%, respectively, which supports the theory of multicentric cancer of the lower genital tract. Prior radiation therapy was rarely associated with increased second gynecologic malignancies (two of 41 patients, 4.9%). Four patients had three gynecologic tumors. (AM J OBSTET GYNECOL 1987;157:261-7.)

Key words: Multiple cancers, gynecology, radiation therapy

Theodore Billroth[1] documented multiple primary cancers in 1889, and the literature now totals more than 30,000 patients with multiple malignancies. This volume of reports represents both an increased interest in such patients and improved patient survival extending years of neoplastic risk. Physicians fascinated and perturbed by patients with multiple malignancies have raised certain questions: (1) Is the patient cancer prone (i.e., is the incidence of a second malignancy greater in patients with cancer than in the general population)? (2) Is there a genetic, immunologic, or environmental factor responsible for multiple primary cancers? (3) Is the prognosis for the patient altered by multiple malignancies? (4) What alterations in management are needed?

Multiple malignancies of the female genital tract have been reported.[2-10] These reports have identified associated pelvic malignancies, and this information has been useful to physicians involved in gynecologic evaluation of patients with cancer and surveillance. Whether these associated malignancies occur by random event or at a statistically increased rate has not been widely studied.

At Ohio State University, a gynecologic tumor registry established in 1939 maintains records on 6489 patients. The first case of multiple malignancies of the genital tract was recorded in 1941. To determine if patients with a gynecologic tumor were at an increased risk of a second gynecologic tumor, we have analyzed 130 cases of multiple malignancies of the genital tract derived from our registry.

From the Departments of Obstetrics and Gynecology, Preventive Medicine, and Biostatistics, The Ohio State University.
Received for publication May 22, 1986; revised October 23, 1986; accepted February 23, 1987.
Reprint requests: Peter G. Rose, M.D., 517 Means Hall, 1654 Upham Dr., Columbus, OH 43210.

Material and methods

One hundred fifty-six cases of multiple malignancies from the gynecologic tumor registry from 1939 through December 1983 were reviewed. Patients with pelvic malignancies of unknown primary site and cancer metastatic to the pelvis were excluded. In addition, to be included in this study, cases had to fulfill the criteria of multiple primary malignancies proposed by Warner and Gates[11] in 1932: (1) Each of the tumors must present a definite picture of malignancy. (2) Each must be distinct. (3) The probability that one is a metastatic lesion from the other must be excluded.

The evaluation of vaginal cancer after cervical cancer poses problems because of similar histologic findings and anatomic location. The following combinations were accepted: (1) cervical carcinoma in situ with subsequent vaginal carcinoma in situ, (2) cervical carcinoma in situ, with subsequent vaginal invasive cancer, (3) cervical invasive cancer with subsequent vaginal carcinoma in situ and (4) cervical invasive cancer with subsequent vaginal invasive cancer occurring more than 5 years after the patient displayed no evidence of disease.

Careful review of the operative and pathologic reports excluded 21 patients who failed to meet either the criteria of Warner and Gates or vaginal cancers after cervical cancer previously proposed. Five patients with histologically different cancers occurring at the same gynecologic site were also excluded. These patients were excluded because of the absence of histologically specific incidence data over the time period of this study. The 5967 registry patients had tumor types suitable for inclusion in this study. The remaining 130

Table I. Incidence of synchronous second malignancies*

Primary	*Secondary*	*Observed/population*	*Expected*	*Observed/expected*	*p<*
Vulvar CIS	Cx CIS	2/52	0.0156	28.21	0.01
Vulvar	Cx CIS	1/203	0.0272	36.76	Not significant
Vulvar CIS	Uterus	1/52	0.0246	40.65	0.05
Vulvar	Cx	3/203	0.0515	58.25	0.01
Vagina	Vulvar	1/74	0.0043	232.57	0.01
Vagina	Cx CIS	2/74	0.0125	160.00	0.01
Vagina	Cx	1/74	0.0178	56.18	0.05
Cx CIS	Vulvar CIS	3/1206	0.0063	476.19	0.01
Cx CIS	Vulvar	1/1206	0.0121	82.64	0.05
Cx CIS	Vagina CIS	3/1206	0.0018	1666.67	0.01
Cx CIS	Vagina	1/1206	0.0038	263.16	0.01
Cx CIS	Uterus	3/1206	0.1613	18.60	0.01
Cx CIS	Uterus NOS	1/1206	0.0226	44.25	0.05
Cx CIS	Ovary	1/1206	0.1439	6.95	Not significant
Cx	Vulvar CIS	1/2818	0.0183	54.64	0.05
Cx	Vulvar	2/2818	0.0828	24.15	0.01
Cx	Uterus	3/2818	1.0019	2.99	Not significant
Cx	Ovary	3/2818	0.7494	4.00	Not significant
Uterus	Cx CIS	3/1112	0.1573	19.07	0.01
Uterus	Ovary	5/1112	0.4225	11.83	0.01
Ovary	Cx CIS	1/502	0.1077	9.29	Not significant
Ovary	Cx	1/502	0.1220	8.20	Not significant
Ovary	Uterus	3/502	0.2108	14.23	0.05
Ovary	Uterus NOS	1/502	0.0351	28.49	Not significant

CIS = Carcinoma in situ; Cx = cervix; NOS = not otherwise specified.
*All lesions are invasive unless specified carcinoma in situ.

cases were divided into synchronous or metachronous tumors. Synchronous tumors were defined as tumors that were either diagnosed concurrent with the primary tumor, discovered at the primary surgical procedure, or occurred within 1 year of the primary tumor diagnosis. A malignant site was designated the primary cancer if it initiated the patient's evaluation or surgical procedure or was chronologically diagnosed first. Metachronous tumors were simply defined as tumors diagnosed greater than 1 year after the primary neoplasm.

To determine whether the second malignancy was statistically associated with the primary malignancy, expected incidence rates for a second malignancy were calculated according to the person-years method[12] for subgroups of patients with the same tumor site and type (invasive versus noninvasive). Incidence rate tables for invasive and noninvasive gynecologic malignancies occurring between 1939 and 1983 were obtained from the Connecticut tumor registry.[13] For synchronous tumors, the cumulative expected incidence rate was calculated by totaling each patient's expected rate at the year of tumor diagnosis. For metachronous tumors, cumulative expected incidence rates were calculated by totaling each patient's expected rate at the year of diagnosis and for each subsequent year the patient was followed. These values were termed standard incidence rates. Patients with multiple malignancies were included in the incidence calculations based on their primary tumor. For this subgroup of patients, follow-up was calculated until the date of diagnosis of a second malignancy. The statistical significance of the calculated ratio, observed to expected second malignancies, was tested with a table of significant factors for the ratio of a poisson variable to its expectation.[14] To determine if prior radiation therapy was a contributing factor to subsequent malignancy, the ratios of standard incidence rates were calculated as described by Gail[15] for subgroups of patients with or without prior radiation therapy for each tumor combination.

Results

Synchronous pelvic malignancies. Forty-five patients had synchronous cancers. Twenty-one patients (47%) had multiple cancers confined to the lower genital tract; 10 (48%) of these patients were initially evaluated for cervical lesions, seven (33%) for vulvar lesions, and four (19%) for vaginal lesions. In situ lesions accounted for at least one malignancy in 66% of these 21 patients. Eleven patients had concurrent uterine and lower genital tract malignancies, 91% of which were cervical neoplasms. In all cases, the cervical and uterine histologic findings were distinct. Of the 15 ovarian tumors, eight were associated with uterine cancer; of these only one was an endometrioid ovarian tumor and one a stromal ovarian tumor, an androblastoma (Table I).

There were three cases of triple malignancies of the

Table II. Incidence of metachronous second malignancies*

Primary	*Secondary*	*Interval (yr)*	*Observed/population*	*Expected*	*Observed/expected*	*p<*
Vulvar CIS	Vagina	2	1/52	0.0035	286.71	0.01
Vulvar CIS	Cx CIS	8	1/52	0.1086	9.21	Not significant
Vulvar	Vagina	9	1/203	0.0167	59.88	0.05
Vulvar	Cx	8	2/203	0.2686	7.45	Not significant
Vulvar	Ovary	12	2/203	0.4010	4.99	Not significant
Vagina	Vulvar CIS	1½	1/73	0.0041	243.90	0.01
Vagina	Vulvar	15	1/73	0.0215	46.51	0.05
Vagina	Cx	7	1/73	0.0192	10.96	Not significant
Vagina	Uterus NOS	1½	1/73	0.0216	46.30	0.05
Cx CIS	Vulvar CIS	7	2/1206	0.1321	15.14	0.05
Cx CIS	Vulvar	12	1/1206	0.2378	4.21	Not significant
Cx CIS	Vagina CIS	4½	12/1206	0.0458	262.01	0.01
Cx CIS	Vagina	10	13/1206	0.0793	163.93	0.01
Cx CIS	Uterus	7	1/1206	4.1544	0.24	Not significant
CX CIS	Ovary	13	1/1206	2.8087	0.36	Not significant
Cx	Vulvar CIS	14	4/2818	0.1764	22.68	0.01
Cx	Vulvar	12	4/2818	0.7579	5.28	0.05
Cx	Vagina CIS	10	7/2818	0.0623	112.36	0.01
Cx	Vagina	12	12/2818	0.2206	54.40	0.01
Cx	Uterus	10	6/2818	9.5784	0.63	Not significant
Cx	Uterus NOS	13	3/2818	1.1638	1.72	Not significant
Cx	Ovary	16	1/2818	6.2425	0.16	0.05
Uterus	Vulvar	17	1/1112	0.5114	1.96	Not significant
Uterus	Vagina	19	2/1112	0.1398	14.31	0.05
Uterus	Cx CIS	4	1/1112	0.9519	1.05	Not significant
Uterus	Cx	20	1/1112	2.1151	0.47	Not significant
Ovary	Uterus	6	3/501	0.9415	3.19	Not significant

CIS = Carcinoma in situ; Cx = cervix; NOS = not otherwise specified.
*All lesions are invasive unless specified carcinoma in situ.

genital tract. One patient had synchronous carcinomas in situ of the vulva, vagina, and cervix. A second patient had synchronous malignant Brenner tumor of the ovary, leiomyosarcoma of the uterus, and carcinoma in situ of the cervix. A third patient initially had synchronous invasive cancers of the vagina and the vulva successfully treated with radiation therapy but died of ovarian carcinoma that occurred 21 years later. Eight patients (18%) had an additional nongynecologic malignancy; bladder (one), breast (three), colon (two), lymphoma (one), and melanoma (one). Twelve patients died of gynecologic malignancies (52% of all patient deaths); and five patients died of nongynecologic malignancies.

Metachronous pelvic malignancies. Metachronous pelvic malignancies occurred in 85 patients (Table II). Cervical cancer was the primary malignancy in 67 patients (79%). Again, malignancies of the lower genital tract predominated; 63 patients (74%) had cancers restricted to this region. Three of the seven patients with vulvar cancer developed cervical cancer at a mean interval of 8 years. Three of the four patients with primary vaginal cancer who developed a second malignancy did so in the lower genital tract. Eleven patients with cervical cancer had vulvar cancers, and 44 patients with cervical cancer had vaginal cancers as the second primary cancer, with a mean interval of 11.8 and 8.5 years, respectively, between the first and second diagnosis.

Endometrial cancer occurred in nine patients at a mean interval of 10 years subsequent to a diagnosis of cervical cancer treated with radiation therapy alone. Three patients with ovarian carcinoma, one of whom had endometrioid histologic findings treated with oophorectomy alone, developed endometrial cancer.

Although in situ lesions made up one of the malignancies in 42 of 63 patients (66%) with multiple primary cancers limited to the lower genital tract, there was no statistically significant difference in the interval between first and second cancers for invasive versus in situ lesions.

One patient with a triple gynecologic malignancy consisting of an adenocarcinoma of the uterus and a squamous cell cancer of the cervix treated surgically developed squamous cell cancer of the vagina 21 years later.

Eight patients (9%) had a third primary cancer outside the pelvis; bladder (two), breast (one), colon (three), larynx (one), and lymphoma (one). One of these patients had four primary cancers involving the cervix, vulva, colon, and larynx, with 27 years between her first and fourth cancer diagnosis.

Table III. Incidence of second malignancies synchronous and metachronous*

Primary	*Secondary*	*Observed/population*	*Expected*	*Observed/expected*	*p<*
Vulvar CIS	Vagina	1/52	0.0035	286.71	0.01
Vulvar CIS	Cervix CIS	3/52	0.0186	27.62	0.01
Vulvar CIS	Uterus	1/52	0.1656	6.04	Not significant
Vulvar	Vagina	1/203	0.0167	59.88	0.05
Vulvar	Cervix CIS	1/203	0.0149	67.11	0.05
Vulvar	Cervix	5/203	0.2686	18.62	0.01
Vulvar	Ovary	2/203	0.0401	4.99	Not significant
Vagina	Vulvar CIS	1/73	0.0041	243.90	0.01
Vagina	Vulvar	2/73	0.0215	93.02	0.01
Vagina	Cervix CIS	2/73	0.0678	29.50	0.01
Vagina	Cervix	2/73	0.0912	21.93	0.01
Vagina	Uterus NOS	1/73	0.0216	45.93	0.05
Cervix CIS	Vulvar CIS	5/1206	0.01321	37.85	0.01
Cervix CIS	Vulvar	2/1206	0.2378	8.41	0.05
Cervix CIS	Vagina CIS	15/1206	0.0458	312.50	0.01
Cervix CIS	Vagina	14/1206	0.0793	176.54	0.01
Cervix CIS	Uterus	4/1206	4.1544	0.96	Not significant
Cervix CIS	Uterus NOS	1/1206	0.2789	3.59	Not significant
Cervix CIS	Ovary	2/1206	2.2087	0.7121	Not significant
Cervix	Vulvar CIS	5/2818	0.1764	28.35	0.01
Cervix	Vulvar	6/2818	0.7579	7.92	0.01
Cervix	Vagina CIS	7/2818	0.0623	112.36	0.01
Cervix	Vagina	12/2818	0.2206	54.40	0.01
Cervix	Uterus	9/2818	9.5784	0.94	Not significant
Cervix	Uterus NOS	2/2818	1.1638	1.72	Not significant
Cervix	Ovary	4/2818	6.2425	0.64	Not significant
Uterus	Vulvar	1/1112	1.5114	1.96	Not significant
Uterus	Vagina	2/1112	0.1398	14.31	0.05
Uterus	Cervix CIS	4/1112	0.9519	1.05	Not significant
Uterus	Cervix	1/1112	2.1151	0.47	Not significant
Uterus	Ovary	5/1112	3.3221	1.52	Not significant
Ovary	Cervix CIS	1/501	0.4253	2.35	Not significant
Ovary	Cervix	1/501	0.4792	2.09	Not significant
Ovary	Uterus	6/501	0.9415	6.37	0.01
Ovary	Uterus NOS	1/501	0.1317	7.59	Not significant

CIS = Carcinoma in situ; NOS = not otherwise specified.
*All lesions are invasive unless specified carcinoma in situ.

Twenty-three patients died of their gynecologic malignancies (69% of all patient deaths), and five deaths were from nongynecologic malignancies.

The cumulative risk for second gynecologic malignancies is summarized in Table III. Malignancies of the lower genital tract statistically increased; 9.6% of patients with vaginal cancer, 4.3% of patients with vulvar cancer, and 1.6% of patients with cervical cancers developed second malignancies of the lower genital tract. In addition, ovarian and uterine malignancies are statistically associated.

Demography. No difference was noted between the age of patients with multiple cancers and those with a single cancer. A review of past medical histories revealed no significant prior diseases compared with the general population. Although 25% of patients reported a family history of cancer, information concerning the degree of kinship was incomplete, and the significance of this is not known.

Prior radiation therapy. None of the patients with synchronous cancer had prior radiation therapy. However, 41 of 85 patients (48%) with metachronous tumors had had prior radiation therapy. Thirty-six of the 41 patients (88%) had the second malignancy 5 years or more after radiation therapy, a time interval considered critical in relation to the carcinogenic risk of ionizing radiation. However, statistical analysis did not associate prior radiation therapy with a subsequent malignancy except in three of the 26 tumor combinations (11.5%) (Table IV). Of these, two tumor combinations (cervical carcinoma in situ: vulvar carcinoma in situ and cervical carcinoma in situ: uterus) were increased by prior radiation therapy, and one tumor combination (cervical: vagina) was decreased. In two of 41 patients (4.9%), radiation therapy was statistically associated with an increase in second malignancy. Of the five metachronous pelvic nongynecologic malignancies (colon three and bladder two), no difference in prior radiation was noted.

Therapy and survival. Ninety-six percent of patients with synchronous multiple cancers and 32% of patients with metachronous multiple cancers received therapy for both malignancies at The Ohio State University. Standard therapies including surgery and radiation

Table IV. Incidence of metachronous pelvic malignancies with and without prior radiation therapy*

Prior radiation	*Primary*	*Secondary*	*Observed/population*	*SIR*	*p*
+	Vulvar CIS	Vagina	0/10	0.0003	
−	Vulvar CIS	Vagina	1/42	0.0032	NS
+	Vulvar CIS	Cx CIS	0/10	0.0071	
−	Vulvar CIS	Cx CIS	1/42	0.0105	NS
+	Vulvar	Vagina	0/61	0.0030	
−	Vulvar	Vagina	1/142	0.0137	NS
+	Vulvar	Cx	0/61	0.0542	
−	Vulvar	Cx	2/142	0.2145	NS
+	Vulvar	Ovary	1/61	0.0690	
−	Vulvar	Ovary	1/142	0.3319	NS
+	Vagina	Vulvar CIS	1/35	0.0015	
−	Vagina	Vulvar CIS	0/38	0.0026	NS
+	Vagina	Vulvar	1/35	0.0065	
−	Vagina	Vulvar	0/38	0.0150	NS
+	Vagina	Cx	1/35	0.0309	
−	Vagina	Cx	0/38	0.0603	NS
+	Vagina	Uterus NOS	1/35	0.0063	
−	Vagina	Uterus NOS	0/38	0.0153	NS
+	Cx CIS	Vulvar CIS	1/97	0.0062	
−	Cx CIS	Vulvar CIS	1/1109	0.1259	0.005
+	Cx CIS	Vulvar	0/97	0.0147	
−	Cx CIS	Vulvar	1/1109	0.2231	NS
+	Cx CIS	Vagina CIS	1/7	0.0019	
−	Cx CIS	Vagina CIS	11/1109	0.0439	NS
+	Cx CIS	Vagina	0/97	0.0049	
−	Cx CIS	Vagina	13/1109	0.0745	NS
+	Cx CIS	Uterus	1/97	0.2521	
−	Cx CIS	Uterus	0/1109	3.9022	0.005
+	Cx CIS	Ovary	0/97	0.1679	
−	Cx CIS	Ovary	1/1109	2.6408	NS
+	Cx	Vulvar CIS	3/2363	0.1534	
−	Cx	Vulvar CIS	1/455	0.0230	NS
+	Cx	Vulvar	4/2363	0.6844	
−	Cx	Vulvar	0/455	0.0735	NS
+	Cx	Vagina CIS	7/2363	0.0546	
−	Cx	Vagina CIS	0/455	0.0077	NS
+	Cx	Vagina	8/2363	0.1975	
−	Cx	Vagina	4/455	0.0231	0.01
+	Cx	Uterus	6/2363	8.5283	
−	Cx	Uterus	0/455	1.0502	NS
+	Cx	Uterus NOS	2/2363	1.0930	
−	Cx	Uterus NOS	0/455	0.0709	NS
+	Uterus	Vulva	0/789	0.3173	
−	Uterus	Vulva	1/323	0.1941	NS
+	Uterus	Vagina	1/789	0.0884	
−	Uterus	Vagina	1/323	0.0513	NS
+	Uterus	Cx CIS	1/789	0.5796	
−	Uterus	Cx CIS	0/323	0.3723	NS
+	Uterus	Cx	0/789	1.3614	
−	Uterus	Cx	1/323	0.7537	NS
+	Ovary	Uterus	2/243	0.3463	
−	Ovary	Uterus	1/258	0.5952	NS

CIS = Carcinoma in situ; Cx = cervix; NOS = not otherwise specified; + = received prior radiation; − = did not receive prior radiation; SIR = standard incidence rates; NS = not significant ($p < 0.05$).

*All lesions are invasive unless otherwise specified.

therapy were used. The presence of multiple or subsequent malignancies did not alter the type of therapy chosen, unless prior radiation precluded radiation.

Mortality rates were determined by the most virulent of the synchronous tumors. Patients with metachronous cancers usually were cured of their primary neoplasm, and the mortality rate was determined by the second malignancy and generally conformed to standard survival figures.

Comment

Despite numerous clinical observations,[3,4,6-10] few authors have statistically evaluated multiple gynecologic malignancies. Arneson and Schellas[4] and Morton and

Villasanta,[4] both of whom studied patients with cervical cancer, noted no increase in second primary malignancies of the genital tract, but they relied on an average patient age and years of follow-up, a statistic now considered obsolete.

Axelrod et al.,[10] using the person-years approach,[13] studied 36 patients with double gynecologic primary malignancies and 42 patients with a gynecologic primary tumor and a nongynecologic second primary tumor. They observed a statistically increased incidence of a second gynecologic malignancy but no increased incidence of nongynecologic tumors except for carcinoma of the cervix and secondary lung or oropharyngeal cancers. This correlation between cancer of the cervix and cancer of the oral cavity, lung, and bladder has been evaluated by Newell et al.[16] and has been shown to be related to cigarette smoking.

Kapp et al.,[9] again applying the person-years statistic to patients with a prior diagnosis of cervical cancer, noted an increased incidence of malignancies of the lower genital tract, as well as lung malignancies, and in small numbers of patients, laryngeal and bone cancer. They noted no increase in nongynecologic pelvic cancers subsequent to prior radiation therapy and concluded that radiation is not an etiologic agent. However, since 93% of their patients received radiation, they were not able to compare different treatment regimens with respect to second primary malignancy rates.

Our results imply that the diagnosis of a patient with a malignancy of the lower genital tract identifies a person at a statistically increased risk for a second such malignancy. Our patients who had 9.6% vaginal, 4.3% vulvar, and 1.6% cervical cancers developed a second malignancy of the lower genital tract. The lack of statistical association with other cancers suggests that the most likely etiologic factor is not a genetic, immunologic, or other systemic factor, since such a defect would result in a generalized increased rate of malignancy. Most likely, the increased risk of a second malignancy of the lower genital tract is related to an environmental factor affecting a field of squamous epithelium as theorized by Newman and Cramer[17] in 1959 and Marcus[2] in 1960. Although not addressed in this study, current evidence suggests the human papilloma virus may be such a carcinogenic environmental factor.[18]

Multiple malignancies of the upper genital tract (uterus and ovaries) were shown to be statistically significant in the occurrence of synchronous tumors but not of metachronous tumors. These findings may simply represent the management of the primary disease with surgical extirpation of the uterus, tubes, and ovaries. In contrast to the findings of McDonald et al.,[6] only one of 12 endometrial tumors was associated with a stromal ovarian neoplasm, and in contrast to expected findings in this patient, it was an androgenic tumor. Recently, Woodruff et al.[19] updated their report on synchronous uterine and ovarian cancers. The authors proposed that based on improved survival such tumor combinations represented a multifocal process rather than metastatic disease.

High-dose therapeutic radiation was originally suggested as a carcinogenic agent in uterine sarcoma by Jacox in 1939, in endometrial carcinoma by Zuspan in 1955, and in pelvic girdle sarcoma by Block in 1962. Subsequent reports supported these observations. Statistical analysis of radiation therapy–induced malignancies is limited to four papers. Czesnin and Wronkowski[6] noted a statistically increased incidence of uterine sarcoma and cutaneous malignancies but no endometrial cancers or pelvic girdle sarcoma. As previously noted, Kapp et al.[8] showed an increased risk of lower genital tract, lung, oropharyngeal and bone malignancies. Both of these authors studied patients with cervical cancer after irradiation, but neither compared their results with patients with cervical cancer treated without radiation. Bailar[20] noted an excess of observed over expected cancers for both irradiation and nonirradiation treatment groups with a relatively greater excess in patients treated surgically when compared with those treated by either radiation or radiation plus surgery. In an international collaborative study, Boice et al.[21] showed that second cancers occurred only slightly more frequently (5%) at all sites than expected in 82,616 patients treated for cervical cancer with radiotherapy. With respect to second gynecologic malignancies, uterine and ovarian cancers were not increased, and other genital malignancies occurred at a statistically increased rate, irrespective of mode of primary therapy (surgery or radiation). We find very little evidence that second malignancies (4.9%) are statistically increased by prior radiation.

In our analysis, the prognosis in patients with multiple gynecologic malignancies does not appear to be worse. Management was only altered if prior radiation therapy had been used.

In conclusion, patients with gynecologic malignancies of the lower genital tract are at increased risk for secondary tumors of the lower genital tract, both initially and subsequently. They should be thoroughly evaluated at initial presentation and require lifelong follow-up.

REFERENCES

1. Bilroth T. Die Allegemeine Chirargische Pathologie und Therape in 51 Voresungen: ein Hunbuch fur Studirende und Artze. Berlin: G Geimer, 1889:908.
2. Marcus SL. Multiple squamous cell carcinoma involving the cervix, vagina and vulva: the theory of multicentric origin. Am J Obstet Gynecol 1960;80:802.
3. Julian CG, Woodruff JD. Multiple anaplasias in the lower genital canal. Am J Obstet Gynecol 1966;95:681.
4. Arneson AN, Schellhas HF. Multiple primary cancers in

patients treated for carcinoma of the cervix. AM J OBSTET GYNECOL 1970;106:115.
5. Morton RF, Villasanta U. New cancers arising in 1,563 patients with carcinoma of the cervix treated by irradiation. AM J OBSTET GYNECOL 1973;115:462.
6. McDonald TW, Malkasian GD, Gaffey TA. Endometrial cancer associated with feminizing ovarian tumor and polycystic ovarian disease. Obstet Gynecol 1977;49:654.
7. Czesnin K, Wronkowski Z. Second malignancies of the irradiated area in patients treated for uterine cervix cancer. Gynecol Oncol 1978;6:309.
8. Kapp DS, Fischer D, Grady KJ, Schwartz PE. Subsequent malignancies associated with carcinoma of the uterine cervix: including an analysis of the effect of patient and treatment parameters on incidence and sites of metachronous malignancies. Int J Radiat Oncol Biol Phys 1982;8:197.
9. Choo YC, Anderson DG. Neoplasms of the vagina following cervical carcinoma. Gynecol Oncol 1982;14:125.
10. Axelrod J, Fruchter R, Boyce J. Multiple primaries among gynecologic malignancies. Gynecol Oncol 1984;18:359.
11. Warren S, Gates O. Multiple primary malignant tumors. Am J Cancer 1932;16:1358.
12. Schoenberg BS, Myers MH. Statistical methods for studying multiple primary malignant neoplasms. Cancer 1977; 40:1892.
13. Connecticut Tumor Registry monograph 45 year report and personal communication. (In press)
14. Bailar JC, Ederer F. Significance factors for the ratio of a poisson variable to its expectation. Biometrics 1964; 20:639.
15. Gail M. The analysis of heterogeneity for indirect standardized mortality ratios. J R Statist Soc A 1978;141:224.
16. Newell GR, Krementz ET, Roberts JD. Excess occurrence of cancer of the oral cavity, lung, and bladder following cancer of the cervix. Cancer 1975;36:2155.
17. Newman W, Cramer JD. The multicentric origin of carcinomas of the female anogenital tract. Surg Gynecol Obstet 1959;108:273.
18. Lancaster WD, Castellano C, Santos C, Delgado G, Kurman RJ, Jensen AB. Human papilloma virus deoxyribonucleic acid in cervical carcinoma from primary and metastatic sites. AM J OBSTET GYNECOL 1986;154:115.
19. Woodruff JD, Solomon D, Sullivant H. Multifocal disease in the upper genital canal. Obstet Gynecol 1985;65:695.
20. Bailar JC III. The incidence of independent tumors among uterine cancer patients. Cancer 1963;16:842.
21. Boice JD, Day NE, Anderson A, et al. Second cancers following radiation treatment for cervical cancer. An international collaboration among cancer registries. J Natl Cancer Inst 1985;74:955.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1987 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 156 (January-June) and Vol. 157 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

The results of cryosurgical treatment of cervical intraepithelial neoplasia at one, five, and ten years

J. L. Benedet, M.D., D. M. Miller, M.D., K. G. Nickerson, M.D., and G. H. Anderson, M.B., B.S.

Vancouver, British Columbia, Canada

The results after cryosurgical treatment of cervical intraepithelial neoplasia at 1, 5, and 10 years are reported. Ninety-four percent of the 1675 patients eligible for assessment at 1 year were successfully treated, with only 5.6% lost to follow-up. After 5 years, 14% of patients were lost to follow-up, but the corrected success rate remained essentially unchanged. No major differences in success rates were noted for the various histologic grades of cervical intraepithelial neoplasia treated. A small but definite percentage of patients developed further disease over the ensuing years, indicating the need for long-term, continued surveillance of these patients. (Am J Obstet Gynecol 1987;157:268-73.)

Key words: Cryosurgery, cervical intraepithelial neoplasia, cryosurgery treatment results

The revival in the early 1970s of colposcopy as a diagnostic technique for the evaluation of patients with abnormal Papanicolaou smears revolutionized the treatment of preinvasive cervical neoplasia. Traditionally, cervical cone biopsy and often simple hysterectomy were the mainstays of treatment. The method selected usually depended on the patient's desire for further pregnancies. Because colposcopy permitted an accurate assessment regarding the nature of the abnormality of the Papanicolaou smear, as well as outlining the extent and location of the lesion, techniques that destroyed local tissue came to be used more frequently. Of these, cryotherapy emerged as the most commonly used method to treat cervical intraepithelial neoplasia, particularly in North America. In 1980, Richart et al.[1] estimated that more than 8000 gynecologists in the United States alone had been enrolled in courses dealing with colposcopy and cryotherapy, which indicates a widespread interest in the use of these techniques.

Numerous publications[2-9] have appeared in the literature that report the results of cryotherapy for the treatment of cervical intraepithelial neoplasia, but few reports[1] are available about its intermediate and long-term effects. Our initial experience[10] with cryotherapy in a selected group of patients was encouraging and led to increased use because of rising incidence rates for preclinical cervical carcinoma in young women.[11] The purpose of this study was threefold: (1) to report our subsequent results, (2) to determine if increased use of this technique led to a change in effectiveness, and (3) to report the treatment results at 5 and 10 years after cryotherapy. We were particularly interested in trying to determine what proportion of patients developed further cervical neoplasia after apparent initial success with this treatment.

From the Department of Obstetrics and Gynaecology, Faculty of Medicine, University of British Columbia, Vancouver General Hospital, and the Divisions of Gynaecologic Oncology and Pathology, Cancer Control Agency of British Columbia.

Received for publication December 11, 1986; revised March 31, 1987; accepted April 9, 1987.

Reprint requests: J. L. Benedet, M.D., Department of Gynaecology, Vancouver General Hospital, 855 W. 12th Ave., Vancouver, B. C., Canada V5Z 1M9.

Material and methods

The records of all our patients treated for cervical intraepithelial neoplasia with cryotherapy at the Vancouver General Hospital since 1973 were reviewed. Although the merits of the cervical intraepithelial neoplasia system of classification for intraepithelial lesions were recognized, we have also continued to report histologic diagnosis by degree of dysplasia to maintain consistent data recording for statistical and analytic purposes at our institution. The correlation between the two systems we have used is as follows: cervical intraepithelial neoplasia I, mild dysplasia; cervical intraepithelial neoplasia II, moderate dysplasia; and cervical intraepithelial neoplasia III, severe dysplasia or carcinoma in situ. All patients had pretreatment colposcopic assessment and directed biopsy. After correlation of the patient's cytologic findings, colposcopic impression, and directed biopsy findings, a treatment recommendation was made. Patients with a squamocolumnar junction that was entirely visible with no endocervical extension of disease were considered candidates for cryosurgical treatment. A Krymed cryosurgery unit (Model MT600) with nitrous oxide as a refrigerant was used in all cases. This equipment

produces a probe-tip temperature of approximately −83° C. The cryosurgical probes used were selected to correspond to the lesion size, and most patients were treated with a mean active freeze time of 3 to 4 minutes. In a few instances, patients with extensive lesions were treated either with two treatment sessions or with several overlapping freezes at a single session. The main criteria used to determine the effectiveness of the freeze at the time of treatment was the extension of the ice ball 3 to 4 mm beyond the periphery of the lesion.

Patient follow-up consisted of visits at 3- to 4-month intervals for colposcopic examination and repeat Papanicolaou smears. Biopsies of any persistent lesions or suspicious areas were also carried out. If the cytologic findings of the patient's cervical smear and the colposcopic appearance of the cervix were normal after 1 year of follow-up (a minimum of three negative smears), she was then discharged from the clinic to the care of her family physician for cervical cytologic examination on a 6-month basis. All Papanicolaou smears in the Province of British Columbia are examined by a single, central cytology laboratory, and further access to patient records and Papanicolaou smear reports was readily available through the laboratory computer system.

Treatment was defined as successful when a patient had normal cervical cytologic and colposcopic findings 1 year after treatment. Recurrent disease was defined as the development of a new squamous cell neoplasm after 1 year of normal cytologic and colposcopic follow-up.

The data were analyzed by means of Pearson's χ^2 test.

Results

The number of patients treated during the two time periods with the success and failure rates, as well as the number and percentage of patients lost to follow-up at 1 year, is presented in Table I. Slightly more than twice as many patients were treated during the second, more recent, and shorter time period, which illustrates the increased use and confidence in cryotherapy as a treatment modality. In addition, the proportion of patients with cervical intraepithelial neoplasia III (severe dysplasia—carcinoma in situ) treated decreased from 71% to 60%. This change was simply because more patients with cervical intraepithelial neoplasia I (mild dysplasia) and II (moderate dysplasia) were treated in the latter time period rather than a decrease in the incidence rate of in situ carcinoma in our population. No apparent difference was noted in the success or failure rates between the two time periods under review.

The age distribution for all patients in the study is listed in Table II. Eighty-three percent of patients were 30 years of age or younger when treated.

Tables III through V present the results of cryosurgical treatment for all patients available for assessment at 1, 5, and 10 years, respectively. The success rates for the various degrees of dysplasia at any time period are similar with no statistically significant differences. The overall success rate at 1 year was 94.3%. The corresponding rates for all patients at 5 and 10 years were 89% and 85%, respectively. At 1 year, only 5.6% of patients were lost to follow-up, but this increased to 28.8% by 10 years.

All patients who were eligible for 5 or more years of follow-up and in whom the treatment was judged to be unsuccessful were analyzed as to the specific years at which they were noted to have recurrent disease. These results are presented in Table VI. Most treatment failures occurred in the first year and are best classified as primary failures, since they probably reflect cryotherapy's inability to eradicate disease in the first instance. It is also likely that several of the patients in whom the treatment failed in the second year were not truly cured of the disease initially, since some had mild cytologic atypicalities on Papanicolaou smears, which led to their continued surveillance through the Colposcopy Clinic and subsequent diagnosis of persistent dysplasia or carcinoma in situ. Table VI also shows cumulative failure rates by year for these patients. Approximately 6% of patients were considered treatment failures by year 1, and over the ensuing 7 years an additional 4.5% of the patients developed further disease.

The rate of new disease per thousand woman-years was calculated as 5.0/1000. This rate is similar not only to that noted for cone biopsy (5.3/1000) in an earlier study,[12] but also to the incidence rate for preinvasive cervical neoplasia in screened women under the age of 40 years in our province (5.41/1000).

The histopathologic diagnosis noted at the time the patients were found to have recurrent disease was then compared with the initial biopsy diagnosis, and these results are shown in Table VII. One patient treated for squamous carcinoma in situ had an abnormal Papanicolaou test at 6 years that showed suspicious gland cells and was found to have invasive adenocarcinoma of the endocervix. The histopathologic diagnosis in 71 of the remaining 80 patients was similar to the initial pathologic diagnosis. In six of the remaining nine patients, the pathologic diagnosis of the recurrence was more than one grade of dysplasia higher than the initial pathologic diagnosis, which indicates possible progression of disease. In three of these patients, the more advanced pathologic diagnosis was noted within the first year of treatment and may represent a diagnostic error rather than true progression. In two patients treated for moderate dysplasia, recurrences were noted at 3 and 5 years and both were carcinoma in situ.

Except for one patient who was admitted to the hos-

Table I. Cryotherapy: Treatment results at 1 year by histologic diagnosis for the time periods 1973 to 1979 and 1980 to 1984

	Patients treated		*Success*		*Failure*	
			1973-1979	*1980-1984*	*1973-1979*	*1980-1984*
Histologic diagnosis	*1973-1979*	*1980-1984*	*No. (%)*	*No. (%)*	*No. (%)*	*No. (%)*
Mild dysplasia (CIN I)	42	111	40 (95)	96 (87)	1 (2)	6 (5)
Moderate dysplasia (CIN II)	109	361	98 (90)	331 (91)	5 (4.5)	14 (4)
Severe dysplasia (CIN III)	136	419	119 (87)	381 (90)	12 (9)	17 (4.1)
Carcinoma in situ (CIN III)	229	268	197 (86)	241 (88)	19 (8)	17 (6.3)
Total	516	1159	454 (88)	1049 (90)	37 (7)	54 (5)

CIN = Cervical intraepithelial neoplasia.

Table II. Patient distribution by age and histologic diagnosis

	Total		*Histologic diagnosis*							
			Mild dysplasia (CIN I)		*Moderate dysplasia (CIN II)*		*Severe dysplasia (CIN III)*		*Carcinoma in situ (CIN III)*	
Age range (yr)	*No.*	*(%)*	*No.*	*(%)*	*No.*	*(%)*	*No.*	*(%)*	*No.*	*(%)*
≤20	188	(11)	14	(9)	53	(11)	70	(13)	51	(10)
21-25	666	(40)	61	(40)	165	(33)	230	(41)	210	(42)
26-30	532	(32)	40	(26)	150	(32)	169	(30)	173	(35)
31-35	220	(13)	24	(16)	72	(15)	68	(12)	56	(11)
>35	69	(4)	14	(9)	30	(6)	18	(3)	7	(1)
Total	1675	(100)	153	(100)	470	(100)	555	(100)	497	(100)

CIN = Cervical intraepithelial neoplasia.

Table III. Cryotherapy: Results of treatment at 1 year

	Patients treated	*Available for assessment*		*Success*		*Failure*	
Histologic diagnosis		*No.*	*(%)*	*No.*	*(%)*	*No.*	*(%)*
Mild dysplasia (CIN I)	153	143	(93.5)	136/143	(95.1)	7/143	(4.9)
Moderate dysplasia (CIN II)	470	448	(95.3)	429/448	(95.7)	19/448	(4.3)
Severe dysplasia (CIN III)	555	529	(95.3)	500/529	(94.5)	29/529	(5.5)
Carcinoma in situ (CIN III)	497	474	(95.4)	438/474	(92.4)	36/474	(7.6)
Total	1675	1594	(95.2)	1503	(94.3)	91	(5.7)

CIN = Cervical intraepithelial neoplasia.
$\chi^2_3 = 5.1518$; $p = 0.16$.

pital 6 days after treatment because of vaginal bleeding caused by an infected cervix, no major complications of treatment were recorded. A degree of menstrual-like cramps was present in virtually all patients during treatment, but this was transient and subsided rapidly on completion of treatment. This was not of sufficient severity in any patient to warrant termination of the procedure. No pretreatment medication with either analgesics, tranquilizers, antispasmodics, or nonsteroidal anti-inflammatory agents was used. Facial flushing also occurred after treatment in most patients but was short-lived.

Lost to follow-up	
1973-1979	*1980-1984*
No. (%)	*No. (%)*
1 (2)	9 (8)
6 (5.5)	16 (4.4)
5 (4)	21 (5)
13 (6)	10 (4)
25 (5)	56 (4.8)

Although many of the patients in this study were known to have had successful uncomplicated pregnancies after treatment, the data recorded for this particular study did not enable an accurate assessment of fertility and obstetric performance after cryotherapy.

Comment

During the past decade, numerous reports[1, 4-10] have appeared in the literature attesting to the validity of the work of Crisp et al.[2, 3] in the use and effectiveness of cryotherapy as a treatment modality for cervical intraepithelial neoplasia. One of the main problems that has been noted is the difficulty in obtaining long-term treatment results because of poor patient compliance for follow-up.

The results of this study indicate that cryotherapy was effective treatment for patients with cervical intraepithelial neoplasia. All but 6% of patients were available for follow-up at 1 year. The results appeared to be equal for the various types of lesions treated, since it did not appear that histologic diagnosis was a major factor in determining success or failure with this method. The relative importance of lesion size or histologic diagnosis as to which is the more important determinant of success is as yet unresolved. Most studies[13-15] have shown that the severity of cervical intraepithelial neoplasia is a factor, but only a few studies have actually assessed lesion size and histologic diagnosis both as independent and covariables.

This study also demonstrated the difficulty in maintaining patient follow-up records over an extended period. Patients with these lesions tend to be young and are often unmarried; with time, they marry, change their names, and move, particularly in times of high unemployment, thus making follow-up extremely difficult. Nonetheless, the results in the 725 patients who were followed up for 5 years were encouraging, since the success rate remained high and only a few patients developed further disease after the first 2 years. Most treatment failures occurred within the first 2 years of treatment, although a small but definite percentage of patients continued to fail after this time. For some patients, these failures are secondary and probably represent a new focus of disease. These findings serve to emphasize that these patients must still be considered at risk for further disease and thus require close surveillance indefinitely.

We believe that the success rate achieved in this series is because of careful patient selection and meticulous technique in carrying out this treatment. No significant endocervical extension should be present, all margins of the lesion seen should be visible, and good probe application is necessary, with an adequate ice ball formed that extends 3 to 4 mm beyond the edge of any lesion identified. It is also imperative to maintain adequate pressure in the system so that a good ice ball is rapidly formed and maintained.

Although no patients in this study developed invasive squamous cell carcinoma during the years under review, caution must be exercised in interpreting these findings, since the natural history of cervical neoplasia suggests a long latent period between in situ and invasive carcinoma in most instances. Sevin et al.[16] and Townsend et al.[17] have reported the occurrence of invasive carcinoma of the cervix in patients treated with cryosurgery. Most of these cases, however, suggest inadequate pretreatment evaluation rather than cryosurgery causing rapid disease progression. These factors underline the need for careful, meticulous, and expert pretreatment assessment if such problems are to be minimized.

The advantages of cryotherapy are numerous; not only is it an extremely effective treatment method for cervical intraepithelial neoplasia, but it is easy to use, is well tolerated by patients, has minimal side effects or complications, requires no analgesia or anesthesia, and is relatively inexpensive. Despite these advantages, enthusiasm for laser therapy appears to be overshadowing the use of cryotherapy, with an ever-increasing number of physicians learning the techniques of laser surgery.

In regional referral centers and large hospital-based treatment clinics, the ability to use all treatment modalities becomes desirable given the number and range of patients managed in such units. Physicians seeing smaller numbers of patients should be encouraged to continue with cryosurgery given the satisfactory results that can be obtained. The additional expense of laser equipment should be carefully weighed against its potential advantages in a given practice situation. Cryotherapy has the additional advantage of being odorless and does not require expensive ancillary equipment for the evacuation of smoke and odor, which is impor-

Table IV. Cryotherapy: Results of treatment at 5 years

Histologic diagnosis	Patients treated	Available for assessment		Success		Failure	
		No.	(%)	No.	(%)	No.	(%)
Mild dysplasia (CIN I)	54	51	(94.4)	45/51	(88.2)	6/51	(11.8)
Moderate dysplasia (CIN II)	207	177	(85.5)	159/177	(89.8)	18/177	(10.2)
Severe dysplasia (CIN III)	254	211	(83.1)	192/211	(91)	19/211	(9)
Carcinoma in situ (CIN III)	328	286	(87.2)	252/286	(88)	34/286	(11.9)
Total	843	725	(86)	648/725	(89.4)	77/725	(10.6)

CIN = Cervical intraepithelial neoplasia.
$\chi^2_3 = 1.1727$; $p = 0.760$.

Table V. Cryotherapy: Results of treatment at 10 years

Histologic diagnosis	Patients treated	Available for assessment		Success		Failure	
		No.	(%)	No.	(%)	No.	(%)
Mild dysplasia (CIN I)	5	2	(40.0)	2/2	(100.0)	0/2	(0.0)
Moderate dysplasia (CIN II)	28	22	(78.6)	19/22	(86.4)	3/22	(13.6)
Severe dysplasia (CIN III)	33	22	(66.7)	20/22	(90.9)	2/22	(9.1)
Carcinoma in situ (CIN III)	59	43	(72.9)	35/43	(81.4)	8/43	(18.6)
Total	125	89	(71.2)	76	(85.4)	13	(14.6)

CIN = Cervical intraepithelial neoplasia.
$\chi^2_3 = 1.446$; $p = 0.695$.

Table VI. Treatment failures by year and initial histologic diagnosis in patients eligible for 5 or more years of follow-up

Yr after treatment	Histologic diagnosis					Cumulative failure rate (%)
	Mild dysplasia (CIN I)	Moderate dysplasia (CIN II)	Severe dysplasia (CIN III)	Carcinoma in situ (CIN III)	Total	
1	3	14	15	18	50	6.8
2	1		3	6	10	7.4
3		3		3	6	8.1
4	2		1	5	8	9.2
5		1		2	3	9.6
6				2	2	10.0
7				1	1	10.2
8		1			1	10.6
9					0	10.6
10					0	10.6
Total	6	19	19	37	81	

CIN = Cervical intraepithelial neoplasia.

tant where space is limited and ventilation and air-conditioning systems are less than ideal.

Schuurmans et al.[18] have reported excellent results with electrocautery as an outpatient treatment modality without anesthesia. Even though this is the simplest and least expensive method, it has been largely overshadowed by cryotherapy. Similarly, we suspect that cryotherapy, in turn, will be replaced by laser surgery.

We extend our appreciation to Mr. Andrew Coldman, Division of Epidemiology, Biometry and Occupational Oncology, Cancer Control Agency of British Columbia, for his assistance with the statistical analysis of the data.

REFERENCES

1. Richart RM, Townsend DE, Crisp W, et al. An analysis of long-term follow-up results in patients with cervical intraepithelial neoplasia treated by cryotherapy. AM J OBSTET GYNECOL 1980;137:823.
2. Crisp WE, Asadourian L, Romberger W. Application of cryosurgery to gynecologic malignancy. Obstet Gynecol 1967:30:668.

Table VII. Histopathologic diagnosis of recurrences in patients eligible for 5 or more years of follow-up

Initital histopathologic diagnosis	*Histopathologic diagnosis of recurrence*					
	Mild dysplasia (CIN I)	*Moderate dysplasia (CIN II)*	*Severe dysplasia (CIN III)*	*Carcinoma in situ (CIN III)*	*Invasive carcinoma*	*Total*
Mild dysplasia (CIN I)	2	1	1	2		6
Moderate dysplasia (CIN II)		15	1	3		19
Severe dysplasia (CIN III)			18	1		19
Carcinoma in situ (CIN III)			2	34	1*	37
Total	2	16	22	40	1	81

CIN = Cervical intraepithelial neoplasia.
*Adenocarcinoma at 6 years.

3. Crisp WE, Smith MS, Asadourian LA, et al. Cryosurgical treatment of premalignant disease of the uterine cervix. Am J Obstet Gynecol 1970;107:737.
4. Kaufman RH, Conner JS. Cryosurgical treatment of cervical dysplasia. Am J Obstet Gynecol 1971;109:1167.
5. Townsend DE, Ostergard DR. Cryocauterization for preinvasive cervical neoplasia. J Reprod Med 1971;6:55.
6. Creasman WT, Weed JC, Curry SL, et al. Efficacy of cryosurgical treatment of severe cervical intraepithelial neoplasia. Obstet Gynecol 1973;41:501.
7. Kaufman RH, Strama T, Norton PK, et al. Cryosurgical treatment of cervical intraepithelial neoplasia. Obstet Gynecol 1973;42:881.
8. Lickrish GM, Fortier M. Conservative management of intraepithelial cervical neoplasia. Can Med Assoc J 1977; 116:641.
9. Popkin DR, Scali V, Ahmed MN. Cryosurgery for the treatment of cervical intraepithelial neoplasia. Am J Obstet Gynecol 1978;130:551.
10. Benedet JL, Nickerson KG, Anderson GH. Cryotherapy in the treatment of cervical intraepithelial neoplasia. Obstet Gynecol 1981;58:725.
11. Benedet JL, Anderson GH, Simpson ML, et al. Colposcopy, conization, and hysterectomy practices: a current perspective. Obstet Gynecol 1982;60:539.
12. Boyes DA, Worth AJ, Fidler HK. The results of treatment of 4389 cases of pre-clinical cervical squamous carcinoma. J Obstet Gynaecol Br Commonw 1970;77:769.
13. Charles EH, Savage EW. Cryosurgical treatment of cervical intraepithelial neoplasia: analysis of failures. Gynecol Oncol 1980;9:361.
14. Ostergard DR. Cryosurgical treatment of cervical intraepithelial neoplasia. Obstet Gynecol 1980;56:231.
15. Hatch KD, Shingleton HM, Austin M, et al. Cryosurgery of cervical intraepithelial neoplasia. Obstet Gynecol 1981;57:692.
16. Sevin B, Ford JH, Girtanner RD, et al. Invasive cancer of the cervix after cryosurgery. Obstet Gynecol 1979;53:465.
17. Townsend DE, Richart RM, Marks E, et al. Invasive cancer following outpatient evaluation and therapy for cervical disease. Obstet Gynecol 1981;57:145.
18. Schuurmans SN, Ohlke ID, Carmichael JA. Treatment of cervical intraepithelial neoplasia with electrocautery: report of 426 cases. Am J Obstet Gynecol 1984;148:544.

Host defense during pregnancy: Neutrophil chemotaxis and adherence

Peter J. Krause, M.D., Charles J. Ingardia, M.D., Linda T. Pontius, B.S., Harry L. Malech, M.D., Tina M. LoBello, B.A., and Eufronio G. Maderazo, M.D.
Hartford and New Haven, Connecticut

Pregnancy is associated with impairment in several immunologic functions that effect the outcome of certain infectious and autoimmune diseases. Previous studies have indicated that pregnant women have decreased neutrophil motility, but results are discordant. We performed a longitudinal study of polymorphonuclear leukocyte chemotaxis using a micropore filter assay and polymorphonuclear leukocyte adherence using a nylon wool column assay in 58 women during pregnancy, delivery, and the postpartum period. Polymorphonuclear leukocyte chemotaxis was decreased significantly in pregnant women during the second (−20%) and third (−17%) trimesters and 1 to 3 months post partum (−18%), but not during the first trimester (−6%) compared with nonpregnant control subjects. The impairment in polymorphonuclear leukocyte chemotaxis appeared to be a cell-associated rather than a humoral defect. Polymorphonuclear leukocyte adherence was decreased significantly in pregnant women during the third trimester (−16%). These data may help explain the increased incidence of infection and amelioration of certain immunologically mediated illnesses during pregnancy. (Am J Obstet Gynecol 1987;157:274-80.)

Key words: Neutrophil, chemotaxis adherence, pregnancy

Pregnancy is associated with suppression of a wide range of immunologic functions that are thought to effect the outcome of certain infectious and autoimmune diseases.[1,2] Studies done in the preantibiotic era suggested that pregnant women had more severe pneumococcal pneumonia than men or nonpregnant women.[3,4] Pyelonephritis, disseminated gonococcal infection, and *Listeria monocytogenes* bacteremia have been reported to be more common in pregnant than nonpregnant women.[5-8] Women with rheumatoid arthritis and other collagen vascular diseases often have an amelioration of their illnesses during pregnancy.[9] Polymorphonuclear leukocytes are thought to play an important role in the pathogenesis of these illnesses. Normal accumulation of polymorphonuclear leukocytes in an inflammatory site is of critical importance for eradication of bacterial infection and is dependent on normal functioning of polymorphonuclear leukocyte adherence and motility. Several in vitro studies suggest that there is impairment of polymorphonuclear leukocyte motility during pregnancy but results are discordant and there is little information on possible etiologic mechanisms.[10-13] We studied polymorphonuclear leukocyte chemotaxis and adherence in 58 women during pregnancy and delivery and 1 to 3 months post partum and in healthy, nonpregnant control subjects. Significant polymorphonuclear leukocyte chemotactic and adherence defects were noted during the second and third trimesters of pregnancy and in the immediate postpartum period. Data are presented suggesting that the chemotactic defects are the result of cellular rather than serum abnormalities.

From the Division of Pediatric Infectious Diseases, Department of Pediatrics, the Department of Obstetrics and Gynecology, and the Division of Infectious Diseases, Department of Medicine, Hartford Hospital, Hartford, and the University of Connecticut School of Medicine and the Division of Infectious Diseases, Department of Medicine, Yale-New Haven Hospital, New Haven, and the Yale University School of Medicine.
Supported in part by Hartford Hospital research free funds and National Institutes of Health grant Nos. AI 19768 and CA 08341.
Received for publication January 16, 1987; accepted March 3, 1987.
Reprint requests: Peter J. Krause, M.D., Department of Pediatrics, Hartford Hospital, 80 Seymour St., Hartford, CT 06115.

Material and methods

Study subjects. Blood was obtained from healthy, pregnant women who attended an obstetric clinic during the first (0 to 14 weeks), second (15 to 28 weeks), and third (29 to 40 weeks) trimesters and 1 to 3 months post partum. Women with chronic illness or taking medication other than vitamins were excluded from the study. Blood was simultaneously obtained and tested in healthy, nonpregnant subjects of comparable age who were taking no medication. Informed consent to obtain blood was obtained in all cases in accordance with hospital institutional review guidelines.

Preparation of polymorphonuclear leukocytes and serum. Blood obtained from study subjects was heparinized with preservative-free sodium heparin (Fisher Scientific Co., Pittsburgh, Pennsylvania; 50 U/ml blood) and sedimented with 6% hetastarch in saline solution

(5 parts whole blood/1 part hetastarch) at room temperature for 15 to 30 minutes. The leukocyte-rich plasma was centrifuged at low speed (500 × g). The pellet was washed with Medium 199 and then resuspended to a concentration of 5×10^5 polymorphonuclear leukocytes/0.7 ml. The chemotactic factors used in these studies were zymosan-activated serum derived from the incubation of healthy, nonpregnant adult serum with zymosan (5 mg/ml serum) at 37° C for 30 minutes or *N*-formyl-methionylleucyl-phenylalanine (FMLP).

Polymorphonuclear leukocyte locomotion assay. A micropore filter assay was employed using modified Boyden chambers (Ahlco Corp., Southington, Connecticut) as described previously.[14] A polymorphonuclear leukocyte suspension of 5×10^5 cells/0.7 ml Medium 199 was placed in the upper compartment and a chemoattractant was introduced into the lower compartment of the Boyden chamber. The lower compartment contained Medium 199 to test chemokinetic responses of 3% zymosan-activated normal serum or 10^{-8} mol/L FMLP to test chemotactic polymorphonuclear leukocyte responses. In other experiments, serum was placed in the upper compartment of the Boyden chamber to test for the possibility of a cell-directed inhibitor.

The polymorphonuclear leukocytes were allowed to partially penetrate a 13 mm diameter, 5 μm pore size cellulose nitrate filter (Schleicher and Schuell, Inc., Keene, New Hampshire) during a 90-minute incubation period at 37° C in a humid, 5% CO_2 atmosphere. The polymorphonuclear leukocytes were then removed, fixed, stained with hematoxylin, cleared with xylene, and mounted on slides. An automated microsectioning counting technique was used to determine the number of polymorphonuclear leukocyte at 10 μm increments from 20 μm to 120 μm into the filter. Because we used a mixed leukocyte suspension, the first 20 μm of the filter was not examined to avoid inclusion of monocytes and lymphocytes in the cell counts. The mean distance (in micrometers) migrated by the polymorphonuclear leukocytes in 90 minutes of incubation time or locomotion index (LI_{20}) was then determined. A mean LI_{20} value of triplicate determinations was calculated for each blood sample. To minimize filter variability, prepurchase testing of samples of filters from various uncut sheets or lot numbers are performed. Filters from a satisfactory lot are used. Because occasional variations between lots of filters are unavoidable, simultaneous daily testing of healthy adults along with study subjects is always carried out.

Polymorphonuclear leukocyte functional subsets

Micropore filter assay. Differences in motility among polymorphonuclear leukocytes were analyzed by the depth that they reached into the filter. Slow-moving polymorphonuclear leukocytes were arbitrarily defined as those traveling 20 to 60 μm into the filter whereas fast-moving polymorphonuclear leukocytes were those traveling 70 to 140 μm into the filter.

31D8 monoclonal antibody. Polymorphonuclear leukocyte functional subpopulations were also defined with use of the mouse monoclonal antibody 31D8, which binds heterogeneously to polymorphonuclear leukocytes and correlates with polymorphonuclear leukocyte chemotaxis. The methods used for 31D8 labeling and analysis have been described previously.[15, 16] In brief, separated polymorphonuclear leukocytes were washed twice in phosphate-buffered saline solution/bovine serum albumin, counted, mixed with 31D8 antibody, and allowed to incubate on ice for 45 minutes. The polymorphonuclear leukocytes were then washed three times in phosphate-buffered saline solution/bovine serum albumin and incubated for 30 minutes on ice with fluorescein isothiocynate–labeled goat-antimouse IgG. The labeled polymorphonuclear leukocytes were washed three more times in phosphate-buffered saline solution/bovine serum albumin and stored on ice, in the dark, and in the absence of CA^{++} or Mg++ until use. Flow microfluorometry was performed with an EPICS C (Coulter, Electronics Inc., Hialeah, Florida) with an argon laser emitting 500 mW at 488 nm. Forward and 90° light scatter was used to identify the polymorphonuclear leukocyte populations from lymphocytes and monocytes. The fluorescence intensity distribution of 10,000 polymorphonuclear leukocytes was determined. The data were presented as the number of cells on the vertical axis and log green fluorescence on the horizontal axis divided into a three-decade log scale consisting of 255 channels.

Polymorphonuclear leukocyte adherence assay. Polymorphonuclear leukocyte adherence was studied because adherence of polymorphonuclear leukocytes to vascular epithelium is the first step in accumulation of polymorphonuclear leukocytes at an inflammatory site. A well-regulated polymorphonuclear leukocyte adherence is also necessary for normal polymorphonuclear leukocyte chemotaxis.[17] A modification of a previously described nylon wool column adherence assay was used to measure polymorphonuclear leukocyte adherence.[17] Forty milligrams of nylon wool (LeukoPak, Fenwal Inc., Ashland, Massachusetts) was packed to a height of 1.5 cm in a tuberculin syringe without the needle or plunger. Separated polymorphonuclear leukocytes were prepared as described above. An aliquot (0.1 ml) of the polymorphonuclear leukocyte solution was removed for determination of the polymorphonuclear leukocyte count while 1.0 ml was allowed to percolate through the nylon column in the syringe. The effluent was collected, a polymorphonuclear leukocyte count determined, and the percent adherence calcu-

Table I. Mean chemokinetic and chemotactic LI_{20} values in 58 pregnant women and 58 nonpregnant control subjects

	Chemotaxis				*Chemokinesis (Medium 199)**	
	*Activated serum**		*FMLP**			
Study period	*PW*	*C*	*PW*	*C*	*PW*	*C*
Trimester 1	47.3 ± 12.1	50.2 ± 11.3	40.6 ± 14.1	51.4 ± 17.5	34.7 ± 7.4	35.9 ± 8.7
Trimester 2	51.4 ± 11.8†	64.2 ± 12.8	45.0 ± 7.7†	54.9 ± 8.8	37.2 ± 7.4	37.0 ± 7.3
Trimester 3	51.6 ± 17.2‡	62.4 ± 14.4	44.8 ± 8.4	48.9 ± 8.4	36.7 ± 8.9	37.9 ± 8.2
Delivery	49.9 ± 13.6†	61.8 ± 15.1	ND	ND	36.5 ± 6.5	41.4 ± 8.9
Post partum	52.6 ± 12.8‡	64.4 ± 18.5	42.0 ± 7.8	50.3 ± 9.0	42.5 ± 11.8	42.4 ± 11.4

PW, pregnant women; C, control subjects, ND, not done.
*Contents of the lower compartment of the Boyden chamber.
†$p < 0.05$ compared with control with the Student t test.
‡$p < 0.01$ compared with control with the Student t test.

lated according to the formula: percent polymorphonuclear leukocyte adherence = ([original polymorphonuclear leukocyte count − effluent polymorphonuclear leukocyte count]/[original polymorphonuclear leukocyte count]) × 100. Each test was run in triplicate.

Statistical analysis. Significance of differences was evaluated by the Student t test. Linear regression analysis was performed according to standard statistical procedure. A p value <0.05 was considered significant.

Results

Fifty-eight healthy pregnant women were enrolled in the study. Eleven women were studied in all three trimesters, 27 women were studied in at least two trimesters, and 20 were studied in one trimester. Thirteen women were studied at delivery and 21 in the postpartum period. Cord blood samples were obtained from the newborn infants of 13 women. Fifty-two women delivered healthy, term infants. Five infants were premature. There was one miscarriage.

Polymorphonuclear leukocyte chemotaxis. Significant impairment in polymorphonuclear leukocyte chemotaxis was noted in the second and third trimesters of pregnancy, during delivery, and in the postpartum period, but not in the first trimester (Table I). The percentage decrease in polymorphonuclear leukocyte chemotaxis in pregnant women during the second trimester compared with nonpregnant control subjects was −20% with activated normal serum and −18% with FMLP used as chemoattractants. Respective values during the third trimester were −17% for activated normal serum and −8% with FMLP. Polymorphonuclear leukocyte chemokinetic values were not significantly different in pregnant women and nonpregnant control subjects. Results of polymorphonuclear leukocyte chemotaxis and chemokinesis were similar in the 11 women studied in all three trimesters compared with the other 47 women studied in only one or two trimesters (Fig. 1). Further studies indicated that decreased polymorphonuclear leukocyte chemotaxis in pregnant women resulted from cellular rather than humoral abnormalities. No difference in the enhancement of polymorphonuclear leukocyte chemokinesis was observed when serum from pregnant women or nonpregnant control subjects was incubated with polymorphonuclear leukocytes in the upper compartment of the Boyden chamber. These data indicate that a cell-directed inhibitor did not contribute to decreased polymorphonuclear leukocyte chemotaxis during pregnancy. Similarly, there was no evidence of chemotactic factor inactivator activity in serum from pregnant women. Generation of complement-derived chemotactic activity was similar in serum from pregnant women and nonpregnant control subjects as shown by similar polymorphonuclear leukocyte chemotactic values when zymosan-activated serum from pregnant (53.3 ± 15.1 μm) or nonpregnant (58.3 ± 14.1 μm) subjects was placed in the bottom compartment of the Boyden chamber.

Polymorphonuclear leukocyte functional subsets

Micropore filter assay. Analysis of the distribution of polymorphonuclear leukocytes at different distances into the micropore filter in both pregnant and nonpregnant women shows a heterogeneous polymorphonuclear leukocyte chemotactic response (Fig. 2). Pregnant women had a higher percentage of polymorphonuclear leukocytes moving 20 to 70 μm into the filter (81%) than had control subjects (69%; $p < 0.01$). The size of the "slow" population of polymorphonuclear leukocytes was significantly different between women in the second and third trimesters and post partum compared with control subjects but not for women in the first trimester. We examined the possibility that this population of "slow" cells was immature polymorpho-

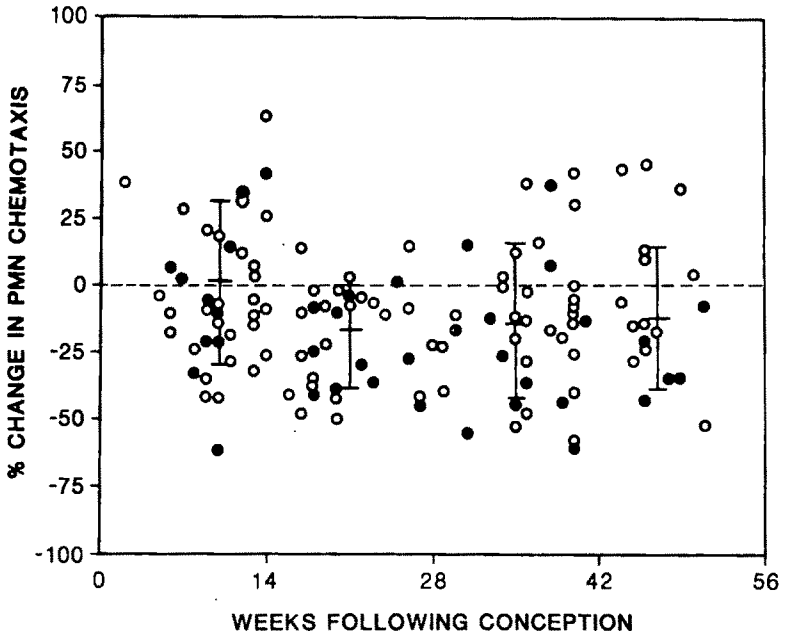


Fig. 1. Percent change in polymorphonuclear leukocyte *(PMN)* chemotaxis in 58 women during pregnancy and the postpartum period compared with 58 nonpregnant control subjects. Mean PMN chemotaxis values using zymosan-activated serum in pregnant women studied in all three trimesters are depicted by *closed circles* and one or two trimesters by *open circles*. The trimesters are delineated as follows: first trimester, 0 to 14 weeks; second trimester, 14 to 28 weeks; third trimester, 28 to 40 weeks, and post partum, 40 to 56 weeks. Mean chemotaxis values ± 1 SD for each trimester are depicted by the *horizontal lines*.

nuclear leukocytes (bands and metamyelocytes) because they are less motile than mature cells. There was a significantly higher percentage of immature polymorphonuclear leukocytes in pregnant women than in nonpregnant control subjects during the third trimester (20.6% ± 8.9% and 5.6% ± 2.3%, respectively; $p < 0.01$) but not during the other two trimesters when compared with control subjects.

31D8 monoclonal antibody–binding assay. To further study the heterogeneous chemotactic response noted with the micropore filter assay, we used immunoglobulin G mouse monoclonal antibody (31D8), which binds heterogeneously to polymorphonuclear leukocytes from the peripheral circulation and correlates with polymorphonuclear leukocyte functional heterogeneity.[15, 16] Polymorphonuclear leukocytes from seven women studied on at least two separate occasions during pregnancy and post partum and nonpregnant control subjects were labeled with 31D8. The majority of polymorphonuclear leukocytes strongly bound 31D8 (31D8 "bright") and generated a highly fluorescent peak. The remaining polymorphonuclear leukocytes weakly bound 31D8 (31D8 "dull") and generated a contiguous, smaller, less fluorescent area to the left of the major peak. 31D8 "bright" cells depolarize, reduce nitroblue tetrazolium, and migrate chemotactically when stimulated with the chemoattractant FMLP, whereas 31D8 "dull" cells fail to respond similarly.[15, 16] The number of polymorphonuclear leukocytes in pregnant women and control subjects that strongly bound 31D8 were quantitated as described previously. No significant differences were noted in 31D8 binding during pregnancy (88.5% ± 5%) compared with control subjects (87.6% ± 4%), nor were any differences noted for any of the trimesters. These data indicate that the differences in the heterogeneous polymorphonuclear leukocyte chemotactic response noted with the micropore filter assay between pregnant women and nonpregnant control subjects was not a result of differences in the amount of 31D8 polymorphonuclear leukocyte antigen.

Polymorphonuclear leukocyte chemotaxis in pregnant women and their newborn infants. Cord blood was obtained from 13 newborn infants whose mothers were enrolled in the study. Mean polymorphonuclear leukocyte chemokinetic and chemotactic values for the newborn infants were 34.3 ± 9.9 μm and 38.6 ± 12.7 μm, respectively, compared with values of 35.8 ± 5.7 μm ($p = 0.63$) and 53.5 ± 8.1 μm ($p = 0.001$), respectively, in their mothers during pregnancy. Linear regression analysis was used to determine whether infants born to mothers with high polymorphonuclear

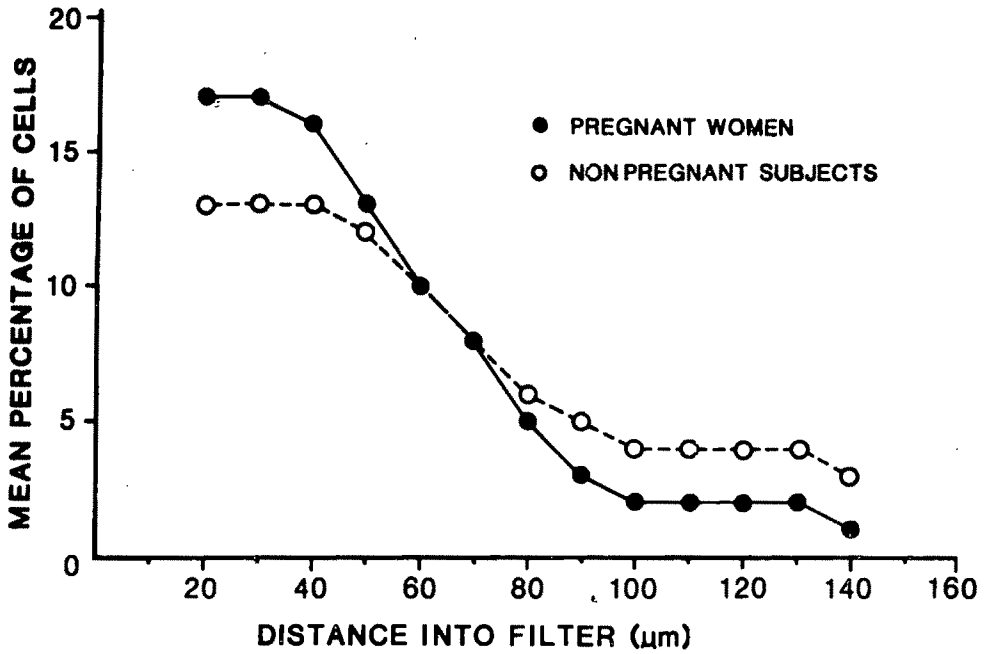


Fig. 2. The heterogeneity of polymorphonuclear leukocyte chemotaxis in the 11 pregnant women studied in all three trimesters of pregnancy and in 11 nonpregnant subjects. The mean percentage of cells that moved into the filter from women during the second trimester of pregnancy are shown.

leukocyte motility values relative to other mothers had high polymorphonuclear leukocyte motility values relative to other infants. No such relationship was found with either polymorphonuclear leukocyte chemokinesis ($r = -0.13$; $p = 0.66$) or chemotaxis ($r = 0.48$; $p = 0.10$).

Polymorphonuclear leukocyte adherence. Mean polymorphonuclear leukocyte adherence values were significantly decreased during pregnancy (76.8% ± 18.4%; $n = 45$) compared with healthy control subjects (86.2% ± 13.8%; $n = 45$; $p < 0.01$). Although mean polymorphonuclear leukocyte adherence values during each trimester and post partum were decreased compared with those of nonpregnant control subjects, it was only during the third trimester that the difference between values in pregnant women (75.0% ± 20.3%; $n = 16$) and nonpregnant control subjects (88.8% ± 12.0%; $n = 16$; $p = 0.03$) were significant.

Comment

The results of this study clearly indicate that there is a significant decrease in polymorphonuclear leukocyte chemotaxis and adherence during the latter part of pregnancy. A well-regulated chemotaxis and adherence enable polymorphonuclear leukocytes to move rapidly from the vascular compartment to the peripheral tissue.[17] Polymorphonuclear leukocytes are thought to be of central importance for both eradication of invasive bacterial pathogens and tissue damage that occurs in several connective tissue disorders. Miles et al.[18] have shown that delay of delivery of polymorphonuclear leukocytes to infected tissue for as little as 2 to 4 hours may prevent the eradication of a focus of infection in spite of eventual accumulation of polymorphonuclear leukocytes as shown by pus formation. The unit of measure of polymorphonuclear leukocyte chemotaxis used in this study (LI_{20}) is the average distance traveled by all the polymorphonuclear leukocytes. Therefore, small numeric differences in the LI_{20} may represent large functional differences in polymorphonuclear leukocyte chemotaxis. Several studies have indicated that lysosomal enzymes and other mediators of inflammation released by polymorphonuclear leukocytes are important causes of rheumatoid inflammation.[19, 20] Our results may therefore help explain the increased severity of several infectious diseases in pregnant women, including gonorrhea, listeriosis, pneumococcal pneumonia, scarlet fever, typhoid fever, diphtheria, septic shock, and pyelonephritis,[1-8] as well as the improvement in symptoms of illnesses such as rheumatoid arthritis and ankylosing spondylitis.[9]

Previous studies of polymorphonuclear leukocyte function during pregnancy have averaged polymorphonuclear leukocyte chemotactic values from different subjects over several months rather than weekly, have not followed the same pregnant women through-

out pregnancy, and have used different assays of polymorphonuclear leukocyte chemotaxis than in our study.[2, 10-13] Nonetheless, our findings of normal polymorphonuclear leukocyte chemotaxis during the first 14 weeks of gestation and decreased polymorphonuclear leukocyte chemotaxis during the subsequent 26 weeks of gestation and for 3 months post partum are similar to those of Bjorksten et al.[10] and Cotton et al.[11] They are also compatible with clinical observations that the greatest severity of certain bacterial infections occur during the latter half of pregnancy.[2-4]

The mechanism(s) of decreased polymorphonuclear leukocyte chemotaxis during pregnancy is unclear. Our results indicate that a cell-associated rather than a serum factor accounts for impaired polymorphonuclear leukocyte chemotaxis in pregnant women. These results are concordant with most previous studies, although the gentle washing used in polymorphonuclear leukocyte isolation procedures may not be sufficient to clear a serum inhibitor. The absence of a diffusable chemotactic inhibitor would help explain why no correlation was noted between the magnitude of impairment of chemotaxis in pregnant women and in their newborn infants. Although previous studies suggest that a cell-associated defect is responsible for decreased polymorphonuclear leukocyte chemotaxis during pregnancy, no specific polymorphonuclear leukocyte defect has as yet been identified in pregnant women.

Previous studies have shown that mature polymorphonuclear leukocytes are not functionally homogeneous. A heterogeneous chemotactic response has been noted among polymorphonuclear leukocytes that appear mature by light microscopy, with approximately 60% to 80% of cells being classified as fast movers and the rest as slow movers.[15, 16, 21] There was a larger percentage of less motile polymorphonuclear leukocytes in pregnant women in the second and third trimesters and in the postpartum period compared with nonpregnant control subjects. This could not be completely explained by differences between the two groups in percentages of immature polymorphonuclear leukocytes because a significant difference in band and metamyelocyte counts was noted only during the third trimester. The monoclonal antibody 31D8 was used to investigate another possible explanation for the increase in less motile polymorphonuclear leukocytes in women during the last two trimesters of pregnancy. Recent studies suggest that the 31D8 monoclonal antibody can detect specific polymorphonuclear leukocyte functional subpopulations derived from different progenitor cells in the bone marrow.[16] No significant difference in antibody binding to polymorphonuclear leukocytes from pregnant women and nonpregnant control subjects was found, however.

Another cell-associated defect that could account for decreased polymorphonuclear leukocyte chemotaxis during pregnancy is polymorphonuclear leukocyte adherence because polymorphonuclear leukocyte chemotaxis is dependent on a well-regulated adherence.[17] Polymorphonuclear leukocyte adherence was decreased in pregnant women during the third trimester. Because polymorphonuclear leukocyte chemotaxis was decreased during the second and third trimesters, impairment of polymorphonuclear leukocyte adherence in pregnant women also does not completely explain the decrease in polymorphonuclear leukocyte chemotaxis.

The results of this study indicate that polymorphonuclear leukocyte chemotaxis is decreased during the second and third trimesters of pregnancy and in the postpartum period whereas polymorphonuclear leukocyte adherence is decreased during the third trimester of pregnancy. These findings might help explain the increased severity of bacterial infections and amelioration of certain collagen vascular diseases during pregnancy. Further studies are needed to elucidate the mechanisms of suppression of polymorphonuclear leukocyte chemotaxis and adherence during pregnancy.

We thank Dr. Charles Woronick, Agnes Reynolds, Charlotte Block, Phyllis Bannon, Claire Eddy, and Patricia Fort for their assistance.

REFERENCES

1. Brabin BJ. Epidemiology of infection in pregnancy. Rev Infect Dis 1985;7:579-603.
2. Larsen B, Galash RP. Host-parasite interactions during pregnancy. Obstet Gynecol Surv 1978;33:297-318.
3. Finland M, Dublin TD. Pneumococcic pneumonias complicating pregnancy and the puerperium. JAMA 1939; 112:1027-32.
4. Rogers ES, Gooch ME. Type I pneumococcus pneumonia: observations from a study of two thousand cases treated with specific serum. New York J Med 1938;38:1369-75.
5. Holmes KK, Counts GW, Beaty HN. Disseminated gonococcal infection. Ann Intern Med 1971;74:979-93.
6. Kass E. Bacteriuria and pyelonephritis of pregnancy. Trans Assoc Am Physicians 1959;72:257-64.
7. Kraus GW, Yen SSC. Gonorrhea during pregnancy. Obstet Gynecol 1968;31:258-69.
8. Nieman RE, Lorber B. Listeriosis in adults: a changing pattern. Report of eight cases and review of the literature, 1968-1978. Rev Infect Dis 1980;2:207-27.
9. Persellin RH. The effect of pregnancy on rheumatoid arthritis. Bull Rheum Dis 1977;27:922-7.
10. Bjorksten B, Soderstrom T, Damber M-G, Von Schoultz B, Stigbrand T. Polymorphonuclear leucocyte function during pregnancy. Scand J Immunol 1978;8:257-62.
11. Cotton DJ, Seligmann B, O'Brien WF, Gallin JI. Selective defect in human neutrophil superoxide anion generation elicited by the chemoattractant *n*-formylmethionylleucylphenylalanine in pregnancy. J Infect Dis 1983;148:194-9.
12. Maltzer MC, Silva J Jr. In vitro defects of phagocyte chemotaxis during pregnancy. J Clin Microbiol 1980;11: 170-3.

13. Takeuchi A, Persellin RH. The inhibitory effect of pregnancy serum on polymorphonuclear leukocyte chemotaxis. J Clin Lab Immunol 1980;3:121-4.
14. Maderazo EG, Woronick CL. Micropore filter assay of human granulocyte locomotion: problems and solutions. Clin Immunol Immunopathol 1978;11:196-211.
15. Krause PJ, Malech HL, Kristie J, et al. Polymorphonuclear leukocyte heterogeneity in neonates and adults. Blood 1986;68:200-4.
16. Seligmann BH, Malech HL, Melnick DA, Gallin JI. An antibody binding to human neutrophils demonstrates antigenic heterogeneity detected early in myeloid maturation which correlates with functional heterogeneity of mature neutrophils. J Immunol 1985;135:2647-53.
17. Krause PJ, Maderazo EG, Scroggs M. Abnormalities of neutrophil adherence in newborns. Pediatrics 1982;69: 184-7.
18. Miles AA, Miles EM, Burke J. The value and duration of defense reactions of the skin to primary lodgement of bacteria. Br J Exp Pathol 1957;38:79-96.
19. Ward PA, Zvaifler NJ. Complement-derived leukotactic factor in inflammatory synovial fluids of humans. J Clin Invest 1971;50:606-16.
20. Weissmann G. Activation of neutrophils and the lesions of rheumatoid arthritis. J Lab Clin Med 1982;100: 322-33.
21. Gallin JI. Human neutrophil heterogeneity exists, but is it meaningful? Blood 1984;63:977-83.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1987 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 156 (January-June) and Vol. 157 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

-DAY* EFFICACY IN A SIMPLE DELIVERY SYSTEM

Now

vaginal cream 2.0%

Femstat® Prefill™

(butoconazole nitrate)



FOR VULVO/
VAGINAL
CANDIDIASIS

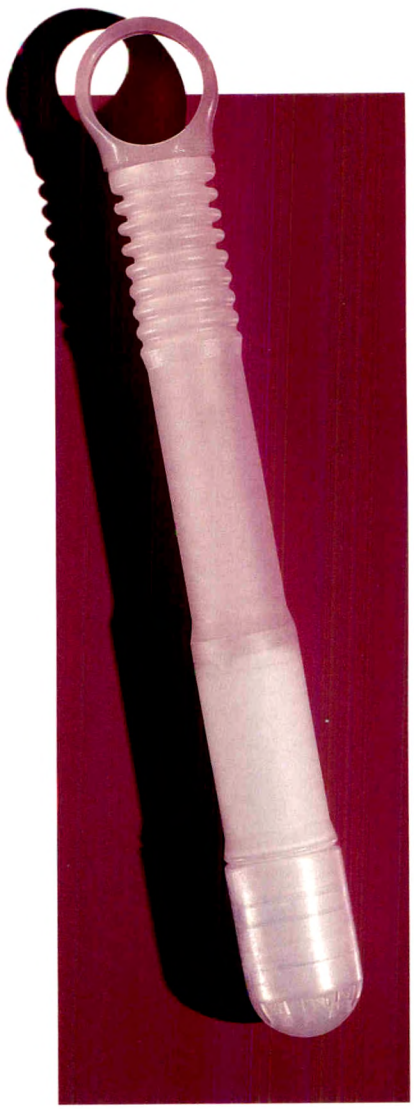
*In non-pregnant patients.
Please see last page for brief summary of prescribing information.

Now

vaginal cream 2.0%

Femstat® Prefill™

(butoconazole nitrate)

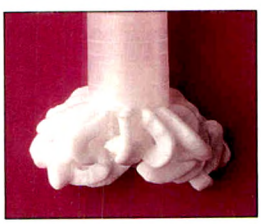


Benefits of 3-Day Cream

- 3-day cure
- Rapid symptomatic relief
- Thorough dispersion

Convenience of Suppositories

- No messy filling procedure
- Disposable applicators
- Unit-dose accuracy
- Minimal leakage



Innovative applicator tip helps ensure complete coverage.

Please see last page for brief summary of prescribing information.

The best of both in a single unique delivery system.



Now

vaginal cream 2.0%

Femstat®

(butoconazole nitrate)

Prefill™

3-DAY EFFICACY
IN A SIMPLE
DELIVERY SYSTEM

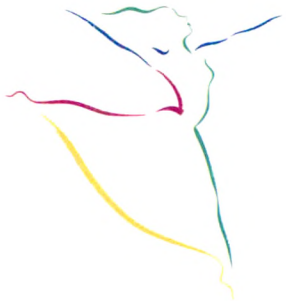
3-DAY EFFICACY IN A SIMPLE DELIVERY SYSTEM

Now

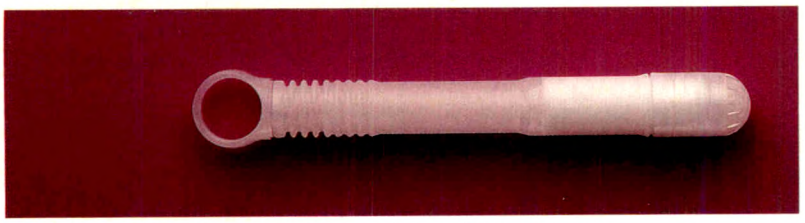
vaginal cream 2.0%

Femstat® Prefill™

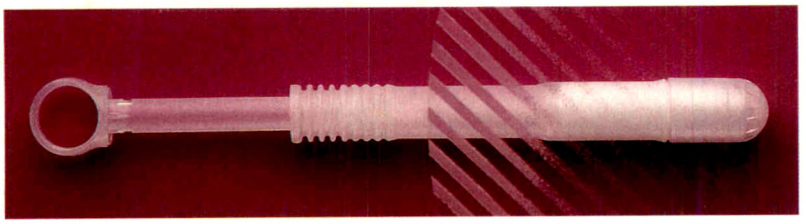
(butoconazole nitrate)



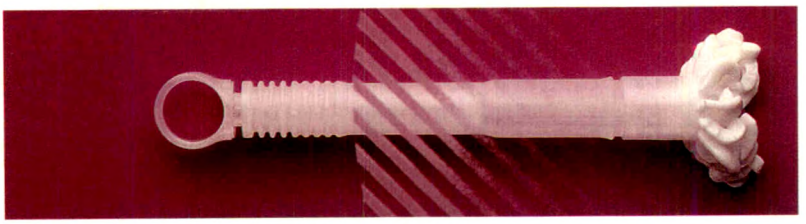
As simple as 1-2-3...



1. Peel back protective foil to remove applicator. Do not attempt to remove special dispersion cap on tip of applicator.

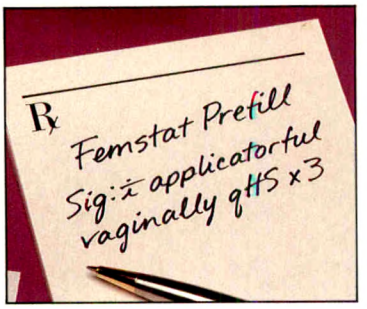


2. Insert applicator into vagina as far as possible without discomfort. Pull back on ring.



3. Push ring all the way forward to deliver medication. Withdraw applicator and discard.





SYNTEX

SYNTEX LABORATORIES INC.
PALO ALTO, CA 94304

© 1987 Syntex Laboratories, Inc.

BRIEF SUMMARY

INDICATIONS:
FEMSTAT® (butoconazole nitrate) vaginal cream 2.0% is indicated for local treatment of vulvovaginal mycotic infections caused by Candida species. Confirm the diagnosis by KOH smears and/or cultures. FEMSTAT can be used with oral contraceptives and antibiotics. It is effective in non-pregnant women and during the second and third trimesters of pregnancy.

CONTRAINDICATIONS:
FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

PRECAUTIONS:
General: If clinical symptoms persist, repeat microbiological tests to rule out other pathogens and confirm the diagnosis. Discontinue drug if sensitization or irritation occurs.

Information for the Patient: Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis: Animal studies have not been done.

Mutagenesis: Mutagenicity studies were negative.

Impairment of Fertility: Animal studies showed no impairment of fertility.

Pregnancy Category C: Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers: Use with caution.

Pediatric Use: Safety and efficacy have not been established.

ADVERSE REACTIONS:
Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

DOSAGE AND ADMINISTRATION:
Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

CAUTION:
Federal law prohibits dispensing without prescription.

February 1987

Varicella zoster antibody testing in the care of pregnant women exposed to varicella

James A. McGregor, M.D.C.M., Saralyn Mark, B.A., Gayle P. Crawford, M.D., and Myron J. Levin, M.D.

Denver, Colorado

Appropriate use of varicella zoster immunoglobulin to prevent or ameliorate maternal or perinatal infection depends on accurate identification of varicella-susceptible women. Detection of fluorescent antibody to varicella zoster virus membrane antigen is an available means for identifying women exposed to varicella who are at risk for infection. During a 5½-year period, 37 pregnant women who had been exposed to varicella and who had a negative or indeterminate history of prior varicella were tested for the presence of varicella zoster virus membrane antigen. Among the 17 women with a negative history, 12 (71%) had a positive test result and five (29%) had a negative test result. Among 20 women with indeterminant histories, 18 (90%) were immune and two (10%) were susceptible. Three additional women who were tested despite a history of varicella had positive varicella zoster virus membrane antigen test results. Overall, 81% of patients with a negative or uncertain history of varicella had serologic evidence of past varicella. One of six untreated seronegative women and one weakly positive woman developed disease. There was no instance of congenital varicella. Expeditious determination of varicella zoster virus membrane antigen or equivalent anti-varicella antibody status in pregnant women exposed to varicella appears to be a rapid, satisfactory method for determining who should promptly receive varicella zoster immunoglobulin passive immunization. (Am J Obstet Gynecol 1987;157:281-4.)

Key words: Varicella, pregnancy, antibody, prevention, serology, immunization

Varicella is an acute, highly contagious, common childhood infection that is increasingly recognized as a potential cause of serious maternal and perinatal morbidity in susceptible pregnant women.[1-3] Varicella in pregnant women may be complicated by preterm labor, pneumonia, and occasionally death.[1-3] Although uncommon, intrauterine infection is associated with stillbirth and stigmata of congenital varicella infection, including mental retardation, microcephaly, blindness, limb hypoplasia, and skin scarring with contractions.[1, 2] Maternal exposure to varicella is not uncommon because of the prevalence of this illness in small children. Past estimates suggest that five cases of varicella or herpes zoster occur per 10,000 pregnancies and that a transplacentally transmitted congenital varicella infection occurs in 5% or less of maternal varicella infections.[3-5] Recent reports indicate that maternal and congenital varicella may be more common than this.[1, 6]

Varicella confers lifelong immunity in immunocompetent hosts. In susceptible individuals, passive immunization with high-titer varicella zoster immunoglobulin given within 96 hours after exposure prevents or ameliorates varicella.[7-9] Although relatively expensive, varicella zoster immunoglobulin is now widely available. Accordingly, passive immunization of susceptible women exposed to varicella is recommended to reduce the risks of maternal and fetal varicella.[9, 10] This represents a change from recommendations made when varicella zoster immunoglobulin was in short supply.

The fluorescent antibody test for varicella zoster virus membrane antigen is a satisfactory means of determining susceptibility to varicella.[11, 12] Recent information suggests that enzyme-linked immunosorbent assay–immunoglobulin G anti-varicella zoster virus serologic testing appears equivalent to varicella zoster virus membrane antigen testing and is easier to perform.[11, 12] Because determination of susceptibility to varicella solely on the basis of a negative history may be unreliable,[8] we evaluated the use of the varicella zoster virus membrane antigen test to determine varicella susceptibility and decide who, among 37 pregnant women with recent exposure to varicella, should receive varicella zoster immunoglobulin.

Material and methods

The exposed pregnant women were seen at the University of Colorado Health Sciences Center between January 1, 1980, and June 13, 1986. Approximately 13,500 deliveries were performed during this period.

From the Department of Obstetrics and Gynecology, University of Colorado Health Sciences Center.

Received for publication November 17, 1986; revised March 16, 1987; accepted April 27, 1987.

No reprints available.

Table I. Correlation of FAMA results in patients with a negative history of varicella

History	N	FAMA			
		Positive		Negative	
		n	%	n	%
Definitely negative	17	12	71	5	29
Indefinite	20	18	90	2	10
Total	37	30	81	7	19

FAMA, varicella zoster virus membrane antigen.

Table II. Correlation of history of varicella, FAMA results, type of exposure, and clinlcal outcome

History/FAMA	Type of exposure		
	Household (child) (n)	Household (nonchild) (n)	Nonhousehold (n)
Negative/negative	3*	0	4†
Negative/positive	9	4	17‡
Positive/positive§	1	2	0

FAMA, varicella zoster virus membrane antigen; V-ZIG, varicella zoster immunoglobulin.

*One patient with varicella pneumonitis, no V-ZIG, >96 hours after exposure.

†One patient given V-ZIG remained well. This is the only patient who received V-ZIG.

‡One patient with positive FAMA who developed mild varicella.

§Includes three patients who underwent FAMA despite history of varicella.

Age, race, socioeconomic status, and obstetric features of these patients were unremarkable. All patients were born in North America. Information concerning past history of varicella, and the timing and type of exposure, was obtained from the patient or other family members. Varicella zoster virus membrane antigen testing was undertaken in 37 women who had had a recent exposure (≤4 days before) and a negative or unknown history of prior varicella. Three additional patients were tested despite a history of varicella. The diagnosis of varicella in the index cases was made on clinical grounds. Varicella zoster virus membrane antigen test procedures, controls, and serologic material were as described elsewhere.[12, 13]

The clinical outcomes of tested women and their newborns were obtained by chart review. Equations derived from Bayes' theorem were used to calculate false negative and positive rates.[13]

Results

Thirty of the 37 (81%) women with negative histories were seropositive, demonstrating forgotten or inapparent prior varicella (Table I). The three women with histories of varicella had positive test results.

Because of restricted supplies, varicella zoster immunoglobulin was not available or recommended for immunocompetent pregnant women during the initial years of this study. Only one seronegative patient received varicella zoster immunoglobulin and she remained well. No infant developed congenital varicella.

Of the 37 patients with negative histories, 12 (32%) had close household contact with infected offspring. Three of these 12 women were actually seronegative (Table II). Among the three seronegative women, only one (33%) developed varicella. Another 21 women were exposed outside their homes to relatives, students, or friends or within the hospital to patients with varicella. Four of these 21 women were seronegative and none developed illness. Thus only one of six (17%) of untreated susceptible pregnant women developed varicella. One woman with a weakly positive test result at 1:2 dilution developed mild, uncomplicated varicella 15 days after a nonhousehold exposure.

A negative history of varicella was unreliable because 81% of such patients had serologic evidence of past varicella. Susceptibility to varicella, as determined by recollection, had a false negative rate of 91% and a false positive rate of 0%. In this clinical setting, varicella zoster virus membrane antigen testing was satisfactory, giving a false negative rate of disease susceptibility of only 16%. A single occurrence of a mild case of varicella in a woman with a weakly positive test result (false positive rate of 50%) was of concern but was not associated with morbidity. These absolute rates are questionable because the numbers are small, exposures were variable, and the population was selected for a negative history of varicella.

Because not all pregnant women with an exposure to varicella were examined serologically during this period, these results should not be considered population based. Nevertheless, the two cases of maternal varicella that occurred at our center during the study were among the 37 tested women who did not have a history of varicella.

Comment

Our findings in pregnant women confirm those of Ross,[8] who showed that approximately 80% of nonpregnant adults with no recollection of varicella are actually immune on the basis of serologic evidence. Ross estimated that only 4% of infections are subclinical. Previously, Ross showed that only 8% of exposed parents with a negative history developed varicella. That

only one of three sero-susceptible women with a household exposure to an infected child developed varicella suggests that pregnant women may not be as susceptible to this infection as are children. Previously, Ross showed that only 8% of exposed parents with a negative history developed varicella. These observations fit with the relatively infrequent occurrence of varicella in pregnant women despite an approximately 10% susceptibility rate in the general population and the anecdotal frequency of maternal exposure to small children with varicella.[14] Subgroups, including immigrants from the Caribbean and the tropics and individuals who were sequestered in childhood, may be at significantly higher risk for acquiring varicella.[14] A negative history of prior varicella is more likely to be reliable within these subgroups.

It should be emphasized that passive immunization in the perinatal setting is aimed primarily at prevention of varicella in the mother and newborn. Because of possible incomplete uptake and transplacental transmission, the newborn of a mother who received varicella zoster immunoglobulin within 5 days before delivery should be passively vaccinated promptly after birth.[9] There is as yet no evidence that passive immunization of varicella virus–susceptible women can prevent intrauterine infection.[9] It is theoretically possible that passive varicella zoster immunoglobulin immunization could modify maternal varicella infection so that it becomes asymptomatic while still allowing transplacental infection.

These results confirm those of Williams et al.[13] showing that varicella zoster virus membrane antigen is sensitive and useful in determining who should receive varicella zoster immunoglobulin. Measurement of varicella zoster virus membrane antigen, or an equivalent test, provides a rapid, practical, and cost-effective means of determining immunity to varicella in exposed pregnant women who give a negative history for prior infection. Direct clinical comparisons with alternative serologic tests, including enzyme-linked immunosorbent assay–immunoglobulin G, remain to be performed.[11, 12]

Although now available for unrestricted use, varicella zoster immunoglobulin remains expensive, costing approximately $75 for a single 125 U vial, with most adult doses totaling at least $375 (one vial for each 10 kg body weight, up to five vials intramuscularly). Laboratory charges for varicella zoster virus membrane antigen are $30 at our institution, so determination of varicella susceptibility is clearly cost-effective because screening can avoid prophylaxis for approximately 80% of exposed individuals with negative or indeterminate histories of past varicella infection. Intramuscular varicella zoster immunoglobulin administration can cause pain and swelling at the injection site, as well as occasional constitutional symptoms and rarely anaphylaxis.[9] Transmission of viral infection, including human immunodeficiency virus (HIV), is not a demonstrated risk.[15]

Serologic testing should be expedited and varicella zoster immunoglobulin given within 96 hours of exposure of seronegative pregnant women to varicella. Although less contagious than varicella, herpes zoster is also a source of varicella zoster virus infection in susceptible individuals. If reliable varicella zoster virus antibody testing is unavailable, pregnant women with a definite negative history of prior varicella infection should be given varicella zoster immunoglobulin. However, it should be realized that the majority of these passive immunizations are unnecessary. Past information suggests that immunocompetent pregnant women with a positive history of past varicella do not require serologic testing or varicella zoster immunoglobulin immunization. In Ross' study[8] of 641 adults exposed to varicella, only one of 475 (0.002%) with a positive history of prior varicella developed disease. As noted, the only cases of maternal varicella seen at our center during the study period did not have a prior history of varicella.

Consideration should be given to routinely obtaining a history of past varicella infection early in pregnancy. Susceptibility of women with a negative history could then be determined serologically and the patient advised; varicella zoster immunoglobulin administration could be expedited if exposure occurs during pregnancy. Availability of a safe and effective varicella zoster virus vaccine would allow for active vaccination of susceptible nonpregnant women, as is done for rubella, and facilitate further reduction of varicella-related morbidity in women as well as their fetuses and newborns.

REFERENCES

1. Paryani SG, Arvin AM. Intrauterine infection with varicella-zoster virus after maternal varicella. N Engl J Med 1986;314:1542.
2. Brunnell PA. Fetal and neonatal varicella-zoster infections. Semin Perinatol 1983;7:47.
3. Harris RE, Rhoades ER. Varicella pneumonia complicating pregnancy: report of a case and review of the literature. Obstet Gynecol 1965;25:734.
4. Sever J, White LR. Intrauterine viral infections. Ann Rev Med 1968;19:471.
5. Siegel M, Fuerst HT, Peress NS. Comparative fetal morbidity in maternal viral disease: a prospective study on rubella, measles, mumps, chickenpox and hepatitis. N Engl J Med 1966;274:768.
6. Borzyskowski M, Harris RF, Jones RWA. The congenital varicella syndrome. Eur J Pediatr 1981;137:335.
7. Brunell PA, Gershon AA, Hughes WT, et al. Prevention of varicella in high risk children: a collaborative study. Pediatrics 1972;50:718.

8. Ross AH. Modification of chickenpox in family contacts by administration of gamma globulin. N Engl J Med 1962;267:369.
9. Committee on Infectious Diseases. Expanded guidelines for use of varicella-zoster immune globulin. Pediatrics 1983;72:886.
10. Immunization Practices Advisory Committee. Varicella-zoster immune globulin for the prevention of chickenpox. MMWR 1984;33:84.
11. Shanley J, Myers M, Edmond B, et al. Enzyme-linked immunosorbent assay for detection of antibody to varicella-zoster virus. J Clin Microbiol 1982;15:208.
12. Enders G. Varicella-zoster virus infection in pregnancy. Prog Med Virol 1984;29:166.
13. Williams V, Gershon A, Brunell PA. Serologic response to varicella-zoster membrane antigens measured by indirect immunofluorescence. J Infect Dis 1974;130:669.
14. Gershon AA, Raker R, Steinberg S, et al. Antibody to varicella-zoster virus in parturient women and their offspring during the first year of life. Pediatrics 1976;58:692.
15. Safety of therapeutic immune globulin preparation with respect to transmission of human t-lymphotrophic virus type III/lymphadenopathy-associated virus infection. MMWR 1986;35:2312.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1987 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 156 (January-June) and Vol. 157 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Sonographic features of the triploid fetus

C. Lockwood, M.D., A. Scioscia, M.D., R. Stiller, M.D., and J. Hobbins, M.D.
New Haven, Connecticut

The sonographic characteristics of six viable triploid fetuses, with gestational ages ranging from 16 to 33 weeks, are presented. Each pregnancy was associated with decreased amniotic fluid volume, a significant lag in biparietal diameter and femur length, and severe head-to-body disproportion. No placental abnormalities were noted on ultrasound and only one placenta demonstrated hydropic change (15% to 20% of villi) on pathologic examination. Since the nonmolar triploid fetus often presents with unexplained intrauterine growth retardation, identification of its characteristic sonographic features allows for karyotype confirmation and the avoidance of inappropriate obstetric management. (AM J OBSTET GYNECOL 1987;157:285-7.)

Key words: Triploidy, partial mole, sonographic findings, head-to-body disproportion

Triploidy is found in 1% of all conceptuses and accounts for 20% of the chromosomal abnormalities identified in spontaneous abortuses.[1] The neonatal incidence of triploidy is 1:2500 to 1:10,000 live births.[1,2] The condition is incompatible with long-term extrauterine survival; rapid postpartum deterioration is the rule. Triploidy associated with partial hydatidiform mole may present as atypical toxemia; alternatively, nonmolar triploidy may be manifested by severe and enigmatic fetal growth retardation. This report describes in detail the sonographic features of the viable triploid fetus in gestations not complicated by obvious partial hydatidiform mole. Recognition of the characteristic sonographic features will enable expeditious karyotype confirmation and appropriate obstetric management.

Material and methods

All diagnoses of triploid karyotype made at the Yale University cytogenetics laboratory between 1984 and 1986 were ascertained. Sources for cell cultures included amniotic fluid obtained by amniocentesis and trophoblasts obtained at the time of pregnancy termination. A total of nine pregnancies had undergone ultrasound examinations, six with viable fetuses and no sonographic evidence of placental molar degeneration and three with sonographic evidence of molar degeneration and either a nonviable hydropic fetus (n = 1) or no identifiable fetal pole (n = 2). Ultrasound evaluations were performed at the Yale University Perinatal Unit and the Bridgeport Hospital Antenatal Testing Unit with a General Electric Model RT3000 (G.E. Corp., Rancho Cordova, California).

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Yale University School of Medicine.
Received for publication November 13, 1986; accepted March 3, 1987.
Reprint requests: Charles Lockwood, M.D., Department of Obstetrics and Gynecology, Tufts University School of Medicine, St. Margaret's Hospital, 90 Cushing Ave., Boston, MA 02125.

Results

Cytogenetic, pathologic, and sonographic data for the six pregnancies accompanied by viable fetuses are presented in Table I. Each pregnancy was associated with decreased amniotic fluid volume, a significant lag in biparietal diameter and femur length, and profound head-to-body (head circumference/abdominal circumference) disproportion. Sonographers also commented on the lack of fetal movement and marked flexion about the spine in Cases 1, 2, and 6.

After amniocentesis, analysis revealed 69,XXX karyotypes for each of the six gestations. No obvious placental cystic change was noted on sonography. Placental pathology reports were available in Cases 1, 2, 3, 5, and 6; hydropic change (15% to 20% of villi) was demonstrated only in Case 3. None of these pregnancies were complicated by toxemia.

In addition, in the three gestations demonstrating obvious placental molar change on ultrasound, the patients presented at 14 to 17 weeks' gestation. In all cases, placental pathologic examination confirmed partial mole. In two cases, a 69,XXY karyotype was identified, with no fetus present in each case (one accompanied by preeclampsia). A hydropic 12-week fetus (crown-rump length) without a heartbeat was identified in a third patient who presented with eclampsia at 14 weeks' gestation; the karyotype was 69,XXX.

Comment

The triploid infant (fetus) is always severely growth retarded with symmetrical shortening of both head size and long bone length.[1] In addition, a significant head-to-trunk disproportion appears to be present.[1] Associated anomalies are variable but can include syndactyly

Table I. Data for six pregnancies with viable fetuses

Case No.	*Evaluation date and indication*	*Karyotype*	*Gestational age**	*Femur length (cm) and gestational age (wk)*	*BPD (cm) and gestational age (wk)*	*Cephalic index (%)*	*Head circumference (cm)*
1	7/25/86 Growth lag	69,XXX	28.5	4.0/23.1	5.7/23.5	69	22.7
2	12/85 Growth lag	69,XXX	22.5	2.7/18.3	4.2/18.6	75	16.0
3	8/84 Oligohydramnios	69,XXX	23.0	3.3/20.4	4.5/19.5	74	17.0
4	4/84 Oligohydramnios	69,XXX	18.0	1.8/15.2	3.1/15.3	75	11.7
5	9/86 Growth lag	69,XXX	33.0	4.7/25.8	6.8/27.6	N/A	25.0
6	10/84 Genetic	69,XXX	16.6	1.5/14.2	2.8/14.5	76	10.5

BPD = Biparietal diameter; HC/AC = head circumference/abdominal circumference ratio; N/A = not available.

*According to last menstrual period.

†*Source:* Campbell S, Thoms A. Ultrasound measurement of the fetal head to abdomen circumference ratio in the assessment of growth retardation. Br J Obstet Gynaecol 1977;84:165.

Table II. Origin of the additional haploid chromosome complement in triploidy

	n	%
Maternal origin (digyny) (n = 50, 31%)		
Failure of first meiotic division	22	44
Failure of second meiotic division	28	56
		100
Paternal origin (diangy) (n = 113, 69%)		
Failure of first meiotic division (dispermy)	30	27
Failure of second meiotic division	6	5
Dispermy	77	68
	113	100

Sources:

Jacobs PA, Szulman AE, Funkhauser J, et al. Human triploidy: relationship between parental origin of the additional haploid complement and development of partial hydatidiform mole. Ann Hum Genet 1982;46:223-31.

Lauritsen J, Bolund L, Friedrich U, et al. Origin of triploidy in spontaneous abortuses. Ann Hum Genet 1979;43:1-5.

Uchida IA, Freeman VCP. Triploidy and chromosomes. Am J Obstet Gynecol 1985;151:65-9.

of the third and fourth digits (61%), congenital heart disease (60%), low-set ears (50%), adrenal hypoplasia (44%), micrognathia (30%), cystic renal dysplasia (26%), colobomas (25%), abdominal wall defects (23%), hydrocephaly–hydranencephaly (18%), neural tube defect (13%), and biliary atresia–gallbladder agenesis (7%).[1] Male infants (69,XXY) have abnormal genitalia (perhaps because of a lack of X chromosome inactivation).[1] Cellular changes include increased nuclear and cytoplasmic volume and a 50% increase in cellular deoxyribonucleic acid content.[1]

The condition arises when an additional haploid complement (n = 23) of chromosomes is present (n = 69). The parental origin of these supernumerary chromosomes is presented in Table II. Approximately 62% of triploid cases have a 69,XXY complement while 37% are 69,XXX; either karyotype may be derived from a maternal or paternal extrahaploid source.[2] The paternally derived 69,XYY karyotype is found infrequently, reflecting a high early loss rate.

The development of placental hydatidiform degeneration–hydropic change is relatively common in triploidy (>50%) while the presence of a partial mole is less frequent.[1] Either trophoblastic abnormality appears to be present in all cases in which there is a paternal origin of the extrahaploid set of chromosomes but is far less common with a maternal origin.[2] The development of toxemia is associated with hydatidiform degeneration–partial mole.

The findings in this small series (confirmed with sonography) illustrate the significant degree of fetal growth retardation present in the triploid fetus. Causes may include an abnormally slow fetal mitotic rate or suboptimal trophoblast nutritional function. While Blackburn et al.[1] described a head-to-trunk discrepancy, implying that the chest is abnormally small, we noted a significant reduction in the abdominal circumference compared with the head circumference. In all five cases where it was recorded, the head circumference/abdominal circumference ratio was greater than the ninety fifth percentile for gestational age (mean 1.53, range 1.89 to 1.31). In the sixth case, marked head-to-body disproportion was observed by the sonographer; however, the abdominal circumference could not be obtained because of marked fetal flexion. Nutritional deficiency with "head sparing," vis-

Abdominal circumference (cm)	*HC/AC and 95th percentile for gestational age†*	*Amniotic fluid volume*	*Placental appearance and grade*	*Maternal complications*	*Comments*	*Placental abnormality*
12.0	1.89 (1.22)	↓	Normal Grade I	None	Marked fetal flexion No fetal movement	No molar changes
9.3	1.72 (1.25)	↓	Normal Grade 0	None	Marked fetal flexion No fetal movement	No molar changes
13.0	1.31 (1.21)	↓	Normal Grade 0	None	No fetal movement	Hydropic degeneration in 15%-20% villi
8.3	1.41 (1.29)	↓	Normal N/A	None	N/A	N/A
19.0	1.31 (1.11)	↓	Normal Grade I	None	Fetal movements present	No molar changes
N/A	N/A	↓	Normal Grade 0	None	Marked fetal flexion	Immature placenta (no molar changes)

ceral organ hypoplasia, or a relatively slower visceral mitotic rate may account for these findings.

Strikingly decreased amniotic fluid volume was present in all six viable gestations. Blackburn et al.[1] reported the occurrence of clinical polyhydramnios in 64% of triploid deliveries but noted that oligohydramnios was also more common. Oligohydramnios, when present, may reflect decreased renal blood flow as a consequence of the intrauterine growth retardation.

An absence of placental hydropic change was confirmed by pathologic examination in four of five placentas examined. Jacobs et al.[2] suggested that the development of trophoblastic hyperplasia in paternally derived triploidy contributes to uterine retention after early embryonic-fetal death. The late-aborting (nonaborting) triploids of maternal origin would be nonmolar. Unfortunately, chromosome polymorphism studies to identify the parental extrahaploid source were not performed. Nonetheless, the lack of hydropic or molar change in four of five placentas studied suggests a predominance of maternal origin.

It is concluded that the combination of early onset (generally ≤28 weeks) fetal growth retardation, extreme head-to-body disproportion, decreased amniotic fluid, and a lack of obvious placental molar change may be indicative of a triploid fetus. These sonographic features should prompt analysis of karyotype and appropriate obstetric intervention if triploidy is confirmed.

The finding of obvious molar change, regardless of fetal status, necessitates immediate analysis of karyotype and, if triploidy is present, expeditious delivery to avoid the development of potentially life-threatening toxemia.

REFERENCES

1. Blackburn WR, Miller WP, Superneau DW, et al. Comparative studies of infants with mosaic and complete triploidy: an analysis of 55 cases. Birth Defects 1982;18(38):251-74.
2. Jacobs PA, Szulman AE, Funkhauser J, et al. Human triploidy: relationship between parental origin of the additional haploid complement and development of partial hydatidiform mole. Ann Hum Genet 1982;46:223-31.

Recurrent small for gestational age birth: Perinatal risks and outcomes

Honor M. Wolfe, M.D., Thomas L. Gross, M.D., and Robert J. Sokol, M.D.
Detroit, Michigan, and Cleveland, Ohio

The risk of recurrent small for gestational age birth, as well as maternal and fetal-neonatal characteristics associated with recurrence, was examined in 174 mothers of consecutively delivered small for gestational age infants followed through an additional 240 livebirths. There was a twofold and fourfold increase in the risk for small for gestational age birth after one and two small for gestational age births, respectively. Although an intervening average for gestational age birth decreased the risk of recurrence, these women remained at increased risk over the general population. Given the history of a previous small for gestational age birth, perinatal risks and outcomes considered individually would not improve the prediction of recurrence. However, the significantly higher frequency of these variables, considered as a group, among mothers with recurrent small for gestational age birth suggests an association with underlying maternal disease, for example, chronic hypertension, substance use and abuse, more severe fetal-neonatal compromise, and recurrent small for gestational age birth. Recurrent small for gestational age birth should initiate a search for persistent, underlying maternal disease. (AM J OBSTET GYNECOL 1987;157:288-93.)

Key words: Intrauterine growth retardation, recurrence, small for gestational age, perinatal risk

Intrauterine growth retardation represents a major source of perinatal morbidity and mortality. Clinical risk assessment remains an important adjunct to sonographic and biochemical methods for the diagnosis and management of impaired growth in utero.[1] Risk factors associated with an initial small for gestational birth have been well delineated.[2] However, of the more than 2000 articles published on intrauterine growth retardation in the obstetric and pediatric literature during the past 20 years, only a small number have addressed the issue of the birth of a small for gestational age infant as a risk for aberrant fetal growth in subsequent pregnancies, that is, risk of recurrence.[3-5] Although a history of a previous small for gestational age birth has been reported to be among the most predictive factors for subsequent intrauterine growth retardation,[3] some of the studies of recurrence have been limited because of sample size[4] and the number of factors examined.[5] Other problems, including the definition used for small for gestational age birth[3] and the growth curve used,[4] may limit the generalizability and comparability of previously reported results.

From the Department of Obstetrics and Gynecology, Hutzel Hospital/Wayne State University, and the Perinatal Clinical Research Center, Cleveland Metropolitan General Hospital/Case Western Reserve University.
Received for publication February 20, 1987; accepted April 28, 1987.
Reprint requests: Honor M. Wolfe, M.D., Department of Obstetrics and Gynecology, Hutzel Hospital/Wayne State University, 4707 St. Antoine Blvd., Detroit, MI 48201.

Our purpose in the present study was to test the hypothesis that the birth of a small for gestational age infant is a substantial risk for subsequent growth retardation. It was further hypothesized that certain perinatal risks and outcomes might increase the risk of recurrent small for gestational age births and thus be useful to identify those mothers of a small for gestational age infant in whom growth retardation will recur.

Material and methods

The 5738 consecutive, singleton livebirths occurring at Cleveland Metropolitan General Hospital from Jan. 1, 1977 through Dec. 31, 1978 were classified according to birth weight for gestational age into birth weight percentile categories with use of data collected prospectively and stored as part of a computer-based perinatal data base.[6] Gestational age was estimated from the Ballard modification of the Dubowitz examination,[7] and birth weight percentile was determined from the Cleveland growth curve. Records of women delivering a small for gestational age (less than the tenth birthweight percentile) infant (index small for gestational age birth) were reviewed. Mothers of an index small for gestational age birth with at least one additional livebirth recorded at that institution from Jan. 1, 1979 through Dec. 31, 1984 were designated as the study sample. Derivation of this study sample is illustrated in Fig. 1.

The study sample was divided, as indicated in Fig. 2, into two groups according to whether the first

Table I. Number of infants after the index small for gestational age birth

No. of deliveries	*No. of patients*
1	174
2	55
3	9
4	1
5	1
Total No. of infants	240

recorded livebirth at the hospital after the index small for gestational age birth was small for gestational age (group I) or not small for gestational age (group II). All subsequent deliveries were similarly classified as small for gestational age or not small for gestational age. The risk for a recurrent small for gestational age birth after the index small for gestational age birth was calculated by determining the frequency of small for gestational age births in the first delivery after the index small for gestational age birth and dividing this by the total number in the study sample (group I divided by [group I + group II]). This value was then divided by the base rate for small for gestational age births observed in our general obstetric population to obtain an estimate of the relative risk.[8] The risk for the birth of an infant weighing less than the twenty-fifth percentile was calculated in a similar fashion by determining the frequency of infants with birth weights less than the twenty-fifth percentile[9] after the index small for gestational age birth and dividing by the base rate for this occurrence in our general population. Risk of recurrence after two consecutive small for gestational age infants was calculated according to the same base rate, as was the relative risk for the birth of a small for gestational age infant in women whose index small for gestational age birth was followed by the birth of an average for gestational age infant.

For patients in the study sample, maternal and neonatal records from the index small for gestational age birth and the next recorded livebirth were reviewed for 14 historical risk factors, nine maternal factors, and five fetal-neonatal factors. These had been chosen a priori as potential risks for recurrence and measures of fetal-neonatal compromise based on previously reported associations with an initial small for gestational age birth. Groups I and II were compared for the frequency of risk factors in the index small for gestational age birth and the next recorded livebirth to evaluate which, if any, risk factors in either pregnancy could be used to identify those mothers of small for gestational age infants who would again deliver a growth-retarded infant.

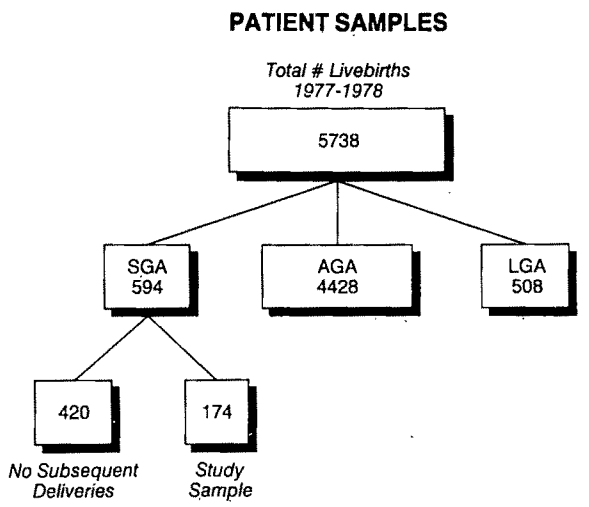


Fig. 1. Birth weight percentile for singleton, livebirths during 1977 and 1978 and derivation of the study sample. *SGA* = Small for gestational age; *AGA* = average for gestational age; *LGA* = large for gestational age.

Statistical analysis was by χ^2 for categoric variables and Student *t* test for continuous variables. Since the hypotheses were directional, one-tailed p values were calculated. To control at least in part for multiple comparisons, $p < 0.01$ was considered significant.

Results

Of the 5738 livebirths in 1977 and 1978, 594 (10.4%) were less than the tenth birth weight percentile, and 1434 (25%) were less than the twenty-fifth percentile. As shown in Fig. 1, of the 594 women with an index small for gestational age birth, 194 were delivered of at least one additional infant at the same institution during the study period. The study group consisted of 174 of these births for which complete perinatal records were available. As shown in Table I, the mothers in the study group were delivered of a total of 240 additional infants during the study period, with a range of one to five deliveries per patient. Comparison of maternal and infant characteristics showed that the study group was a highly representative subgroup of the 594 small for gestational age deliveries during 1977 and 1978 (Table II).

The birth weight percentile categories of infants born to the study group after the index small for gestational age birth are shown in Fig. 2. Fig. 3 summarizes the relative risk for the birth of an infant weighing less than the tenth and twenty-fifth percentile based on past obstetric history. Small for gestational age births recurred in 22.4% (39/174) (group I/group I + group II) of women in the study group after the index small for gestational age birth, resulting in a relative risk for the birth of a small for gestational age in-

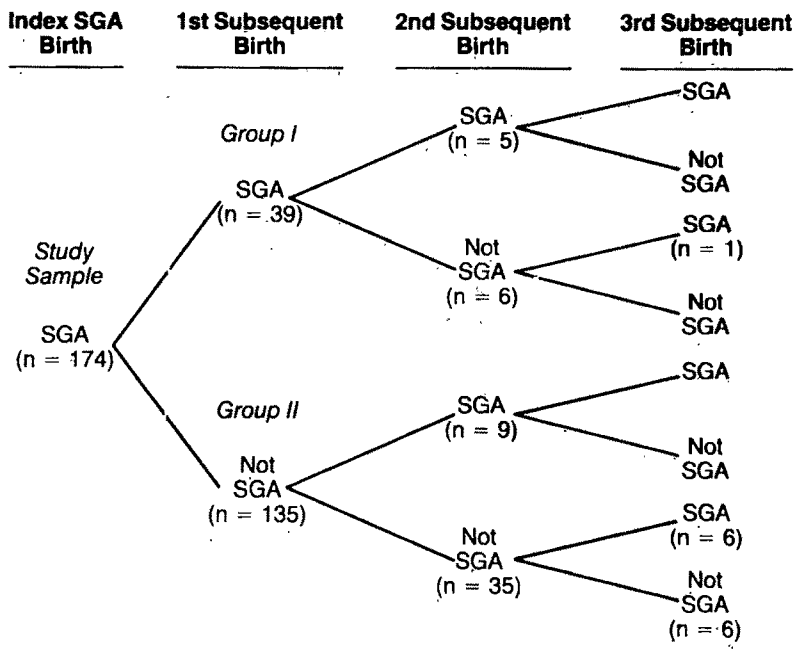


Fig. 2. Birth weight percentile for the study sample in livebirths subsequent to the index small for gestational age birth. *SGA* = Small for gestational age.

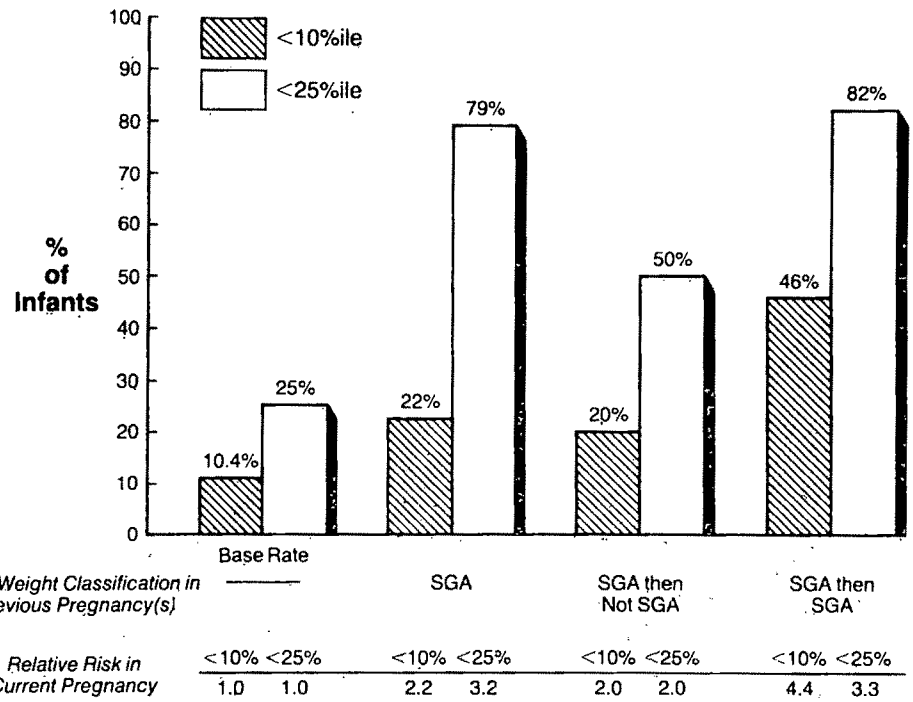


Fig. 3. Relative risk for the birth of an infant less than the tenth and twenty-fifth birth weight percentile based on birth weight classification in previous pregnancies. *SGA* = Small for gestational age.

fant of 2.2 times (22.4/10.4). This was significantly higher than that for the general population ($\chi^2 = 27.3$, $p < 0.001$). Among the study group mothers, 79% (137/174) were delivered of infants weighing less than the twenty-fifth percentile (relative risk, 3.2 times). The relative risk for a small for gestational age birth after two small for gestational age births (4.4 times) was also increased significantly over that for the general pop-

Table II. Comparison of maternal and infant characteristics between the study group and all mothers of small for gestational age infants during the index years 1977 through 1978

		All SGA (n = 594)	*Study group (n = 174)*
Age	Mean	21.8 ± 5.4	20.0 ± 4.2
	Range	13 – 45	13 – 40
Gravidity	Mean	2.4 ± 1.7	2.3 ± 1.6
	Range	1 – 14	1 – 14
Parity	Mean	0.83 ± 1.2	0.69 ± 1.2
	Range	0 – 10	0 – 10
Dubowitz examination	Mean	38.8 ± 2.4	38.8 ± 2.6
	Range	26 – 43	27 – 43
Birth weight	Mean	2337 ± 432	2358 ± 422
	Range	540 – 2863	680 – 2860

Table III. Comparisons of maternal and infant characteristics between group I (mothers with recurrent small for gestational age births) and Group II (mothers without recurrent small for gestational age births) in the index SGA birth

	Group I (n = 39)	*Group II (n = 135)*	p
Maternal characteristics			
Age	21.2 ± 4.6	19.7 ± 4.1	NS
Gravidity	2.60	2.3	NS
Parity	0.6	0.7	
Gestational age at first antenatal visit	16.5	17.1	NS
Prepregnancy weight	124 ± 27	122 ± 25	NS
Pregnancy weight gain	22 ± 13	21 ± 13	NS
Medical			
Hematocrit screening	35.1	34.5	NS
Hematocrit labor	34.4	34.1	NS
Pulmonary disease	5%	1%	NS
Renal disease	2.4%	1.5%	NS
Blood pressure			
First antenatal visit	113/65	109/64	NS
Maximum antepartum	121/70	120/69	NS
Maximum intrapartum	136/80	130/80	NS
Substance use/abuse			
Alcohol abuse	2.4%	1.5%	NS
Tobacco	76%	49%	<0.001
Drug abuse	7.3%	2.2%	NS
Obstetric			
Pregnancy-induced hypertension			
Mild	2.4%	1.0%	NS
Severe	2.4%	1.0%	NS
History of previous stillbirth	0%	2.0%	NS
Pediatric gestational age	38.7 ± 2.7	38.9 ± 2.5	NS
Birth weight	2327 ± 424	2376 ± 418	NS

ulation ($\chi^2 = 14.5$, $p < 0.001$). The increased risk for a small for gestational age birth when the index small for gestational age birth was followed by an average for gestational age birth was less than that after one or two small for gestational age births but remained higher than that for the general population (2.0 times).

Comparisons between groups I and II for risks associated with the index small for gestational age birth are shown in Table III, and those that occurred in the first subsequent pregnancy are seen in Table IV. Few factors differentiated those mothers in whom growth retardation recurred from those without this problem. However, there was a trend toward an increased frequency of medical complications and substance use and abuse in the index small for gestational age birth among mothers with recurrent small for gestational age births. Statistical significance was reached on individual variables for tobacco only ($p < 0.001$). Similar trends were present in the first subsequent delivery, with mothers in group I showing significantly higher systolic blood pressures, a greater than sixfold increase in alcohol use, and a twofold increase in illicit drug use.

Table IV. Comparison of maternal and infant characteristics between group I (mothers with recurrent small for gestational age birth) and Group II (mothers without recurrent small for gestational age birth) in the first pregnancy subsequent to the index small for gestational age birth

	Group I (n = 39)	*Group II (n = 135)*	p
Maternal characteristics			
Age	24.9 ± 4.7	22.5 ± 4.3	NS
Gravidity	3.8	3.5	NS
Parity	1.6	1.5	NS
Gestational age at first antenatal visit	16.1	17.1	NS
Prepregnancy weight	130 ± 36	126 ± 27	NS
Pregnancy weight gain	17.4 ± 11.4	21.7 ± 11.7	NS
Medical			
Hematocrit screening	35.0	34.8	NS
Hematocrit labor	34.1	34.1	NS
Pulmonary disease	5%	1%	NS
Renal disease	2.4%	1.5%	NS
Blood pressure			
First antenatal visit			
Systolic	112	107	<0.01
Diastolic	67	63	NS
Maximum antepartum			
Systolic	122	115	<0.003
Diastolic	69	67	NS
Maximum intrapartum			
Systolic	129	125	NS
Diastolic	78	77	NS
Substance use/abuse			
Alcohol abuse	13%	2%	NS
Tobacco	56%	49%	NS
Drug abuse	3%	1.5%	NS
Obstetric			
Preeclampsia			
Mild	0	1.5%	NS
Severe	0	0	NS
History of previous stillbirth	3%	1%	NS
Pediatric gestational age	38.6 ± 2.9	38.2 ± 2.8	NS
Birth weight	2308 ± 480	2854 ± 588	<0.001

Five parameters of fetal-neonatal outcome (birth weight, fetal distress, need for intrapartum fetal scalp pH sampling, meconium, hypoglycemia, and polycythemia) were compared between groups I and II in the index small for gestational age birth. Differences in mean birth weight did not reach statistical significance. Infants born to mothers with recurrent small for gestational age births (group I) had a fivefold increase (10% versus 2%) in the need for intrapartum fetal scalp pH sampling, a 3.8-fold increase (15% versus 4%) in the frequency of fetal distress, a 3.3-fold increase (5% versus 1.5%) in the frequency of neonatal hypoglycemia, and a 1.5-fold increase (15% versus 10%) in the frequency of meconium. Polycythemia was more frequent (9% versus 7.5%) among infants in group II. Analysis by goodness of fit χ^2 for nine maternal factors (screening hematocrit, pulmonary disease, renal disease, screening blood pressure, alcohol use and abuse, tobacco use, drug abuse, preeclampsia, and a history of a previous stillbirth) and five fetal-neonatal factors (birth weight, fetal distress, polycythemia, hypoglycemia, and meconium) in the index small for gestational age birth hypothesized a priori to be associated with recurrence and revealed that 12 of 14 differences were in the expected direction, that is, the risks and adverse outcomes were associated with recurrent small for gestational age births ($\chi^2 = 7.14$, $p < 0.01$). In the first subsequent pregnancy, two of nine maternal differences were in the expected direction ($\chi^2 = 4.45$, $p < 0.04$). Fetal and neonatal characteristics were not compared in this pregnancy since the two groups had different birth weight classification (small for gestational age versus average for gestational age).

Based on the number of livebirths at the institution from 1979 through 1984 and the women in our study, we estimate that mothers of small for gestational age infants accounted for a minimum of 4% of the small for gestational age births in this population.

Comment

Our finding of a twofold and fourfold increase in small for gestational age birth after one and two pre-

vious small for gestational age infants(s), respectively, confirms the assertion that a history of a previous small for gestational age birth is an important risk for intrauterine growth retardation.[5] Given a history of a previous small for gestational age birth, individual perinatal risks and outcomes do not appear to enhance the predictability of recurrence. When considered as a group, however, our results suggest an association among underlying maternal disease, more severe perinatal compromise, and recurrent small for gestational age birth.

The present study has several strengths, including a homogeneous, single-center population, with the growth curve used derived from previously delivered infants from the same institution. It is also among the first large studies that addressed the use of multiple maternal and fetal-neonatal characteristics for the identification of the risk of small for gestational age recurrence. However, since our study did not include maternal heights, our ability to estimate the relative contribution of constitutional and environmental factors to birth weight percentile was limited.

Previous authors have examined risk factors associated with recurrence. Tejani[4] reported a recurrence rate of 28% in 28 mothers of small for gestational age infants followed through an additional 32 deliveries. She concluded that the likelihood of recurrence is higher in women with severe pregnancy-induced hypertension, chronic hypertension, or drug abuse. Visser et al.[10] confirmed this finding in patients with recurrent, early (<34 weeks) intrauterine growth retardation. Neither Visser et al. nor Tejani, however, performed statistical evaluation of their results, and selection bias may have been present. Nonetheless, the current results are in general agreement with their findings of a trend toward increased medical complications among mothers with recurrent small for gestational age births.

In their study of more than 1000 small for gestational age pregnancies, Patterson et al.[5] confirmed that a synergistic effect between hypertension and a history of a previous small for gestational age infant increased the risk of recurrent growth retardation. Our data, which examined additional medical complications, including renal disease, pulmonary disease, and alcohol use and abuse, are in agreement with their findings. Similar birth weight and gestational age in groups I and II in this study are not consistent with the conclusion of Patterson et al. that recurrence is related to the severity of intrauterine growth retardation, at least as reflected in birth weight percentile. Nonetheless, our data on additonal fetal-neonatal parameters lend support to the supposition that the risk for recurrent intrauterine growth retardation may be related to the severity of the underlying problem.

The large number of infants in this study whose birth weights were less than the twenty-fifth percentile after a small for gestational age birth suggests that fetal growth, although not meeting the criterion for a small for gestational age birth, continues to be suboptimal.

In conclusion, a history of a small for gestational age birth is a major risk for growth retardation. This study confirms a substantial recurrence rate and further estimates that a minimum of 4% of small for gestational age births are attributable to recurrence. Although it appears that growth retardation recurs more frequently among mothers with medical complications, all women with a history of a small for gestational age birth should be considered at increased risk in subsequent pregnancies. In addition, the presence of recurrent small for gestational age births should alert the clinician to the possibility of persistent underlying abnormalities such as renovascular disease and substance use and abuse that might not be apparent in the current pregnancy. Because preceding small for gestational age and preterm low birth weight pregnancies have different risks in the perinatal period, obstetric history and antenatal risk scoring should include not only birth weight but also gestational age and birth weight percentile.

REFERENCES

1. Gailbraith RS, Karchmar EJ, Piercy WN, et al. The clinical prediction of intrauterine growth retardation. Am J Obstet Gynecol 1979;133:281-6.
2. Scott A, Moar V, Ounsted M. The relative contributions of different maternal factors in small-for-gestational age pregnancies. Eur J Obstet Gynaecol Reprod Biol 1981; 12:157-61.
3. Bakketeig LS, Hoffman HJ, Harley EE. The tendency to repeat gestational age and birth weight in subsequent births. Am J Obstet Gynecol 1979;135:1086-103.
4. Tejani NA. Recurrence of intrauterine growth retardation. Obstet Gynecol 1982;59:329-31.
5. Patterson RM, Gibbs CE, Wood RC. Birth weight percentile and perinatal outcome: recurrence of intrauterine growth retardation. Obstet Gynecol 1986;68:464-8.
6. Sokol RJ, Rosen MG, Stojkov J, et al. Clinical application of high risk scoring on an obstetric service. Am J Obstet Gynecol 1977;28:652-61.
7. Ballard JL, Novak KK, Driver M. A simplified score for assessment of fetal maturation of newly born infants. J Pediatr 1979;95:769-74.
8. Lilienfeld AM, Lilienfeld DE. Foundations of epidemiology. 2nd ed. New York: Oxford University Press, 1980.
9. Seeds JW. Impaired fetal growth: definition and clinical diagnosis. Obstet Gynecol 1980;64:303-10.
10. Visser GH, Huisman A, Saathof PW, Sinnige HA. Early fetal growth retardation: obstetric background and recurrence rate. Obstet Gynecol 1986;67:40-6.

Successful fetal therapy for cystic adenomatoid malformation associated with second-trimester hydrops

Steven L. Clark, M.D., Dennis J. Vitale, M.D., Stephen D. Minton, M.D., Ronald A. Stoddard, M.D., and Patty L. Sabey, R.N.
Provo, Utah

Fetal hydrops secondary to cystic adenomatoid malformation was detected in a second-trimester fetus. In utero thoracoamniotic shunt placement resulted in resolution of the hydrops. At term, there was no evidence of pulmonary hypoplasia. (AM J OBSTET GYNECOL 1987;157:294-5.)

Key words: Cystic adenomatoid formation, second trimester hydrops, fetal therapy

Cystic adenomatoid malformation is an uncommon congenital pulmonary anomaly that may result in fetal hydrops, pulmonary hypoplasia, and fetal or neonatal death.[1,2] When associated with early fetal hydrops, this condition has been considered to be uniformly fatal.[1] We present here the first reported case of successful in-utero treatment of cystic adenomatoid malformation associated with massive second trimester hydrops.

Case report

A 36-year-old woman, gravida 6, para 2, abortions 3, underwent ultrasonic examination to document gestational age, revealing a single viable fetus and biometric parameters consistent with 20 weeks' gestation. Real-time ultrasonographic examination revealed that the fetal thoracic cavity was occupied by a single cystic lesion measuring 10 by 6 by 6 cm (Fig. 1). The fetus was also noted to be grossly hydropic with massive ascites and edema of the scalp. Detailed fetal anatomic survey revealed no evidence of additional anatomic malformation. The thoracic lesion was felt to represent cystic adenomatoid malformation versus a remnant cyst of neurogenic or enteric origin. After approval by a multidisciplinary review panel, informed consent was obtained, and the fetus underwent simple transthoracic aspiration of the cyst and genetic amniocentesis. The cyst was completely emptied and when decompressed allowed documentation of normal intrathoracic and cardiac anatomy. Chemical analysis of the aspirated fluid revealed it to be serous.

After 48 hours the cyst was noted to be reexpanding, its dimensions measuring 6 by 8 by 6 cm, and intrathoracic shunting was performed. A 13 cm section of Fr. 6.5 polyethylene tubing was fashioned in a "double pigtail" pattern. The catheter was introduced over a 21 cm 18-gauge pencil-tip needle/stylette combination under local anesthetic, under direct and continuous ultrasound observation (General Electric RT 3000 real-time unit, 5 mHz linear transducer) into the posterior subscapular region of the right fetal chest and into the cyst. The needle was then withdrawn leaving the proximal coiled end extrathoracically within the amniotic cavity. Prompt and complete drainage of the cyst was observed. The fetus tolerated this procedure well. Throughout the next 17 weeks of gestation, the patient underwent weekly ultrasonography. Within 3 weeks of shunt placement evidence of ascites, pleural and pericardial effusions, and edema of the scalp had completely resolved, and fetal activity was noted to increase. At 37 weeks' gestation the patient underwent amniocentesis for documentation of fetal pulmonary maturity and primary cesarean section. A living female with Apgar scores of 8 and 9 and a weight of 2680 gm was delivered. The infant cried vigorously immediately after delivery, had normal blood gas values, and required neither ventilatory support nor supplemental oxygenation. At the time of delivery the extrathoracic portion of the shunt lumen appeared to be occluded with vernix; however, fluid was noted to be leaking from the fetal thoracic cavity around the shunt. The infant was taken to the operating room the same day (Fig. 2). A thoracotomy was performed and the distal end of the tube was found to lie within a cystic cavity contained within the right middle pulmonary lobe. The catheter was removed under direct surgical visualization, and the infant underwent resection of the right middle lobe. Both right upper and lower lobes were normal in size and consistency. They inflated readily intraoperatively and showed no clinical evidence of hypoplasia. Subsequent pathologic examination confirmed the diagnosis of macrocystic adenomatoid malformation. After surgery the infant did very well and remained on room air. At the time of this re-

From the Utah Valley Regional Perinatal Center.
Received for publication August 7, 1986; revised December 2, 1986; accepted February 6, 1987.
Reprint requests: Steven L. Clark, M.D., Utah Valley Regional Medical Center, 1034 N. 500 W., Provo, UT 84603.

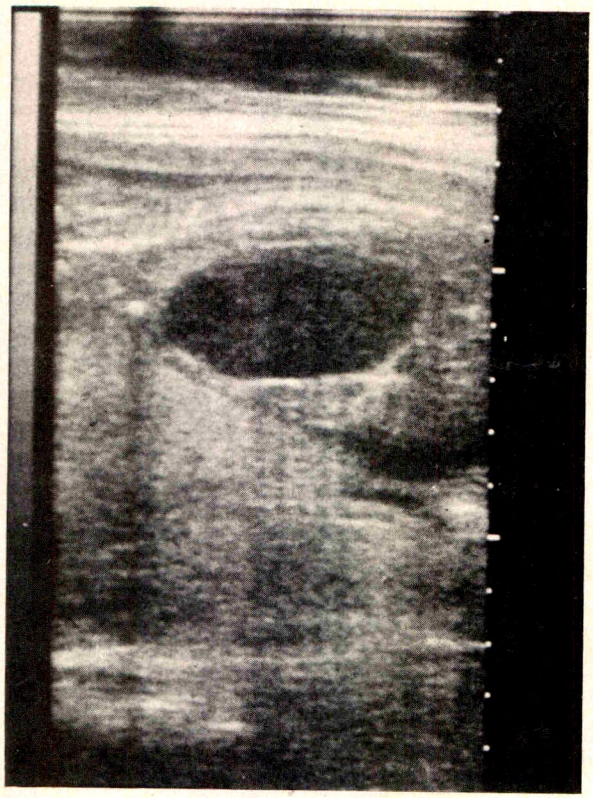


Fig. 1. Cystic adenomatoid malformation. Normal intrathoracic structures are replaced by large, unilocular cyst.

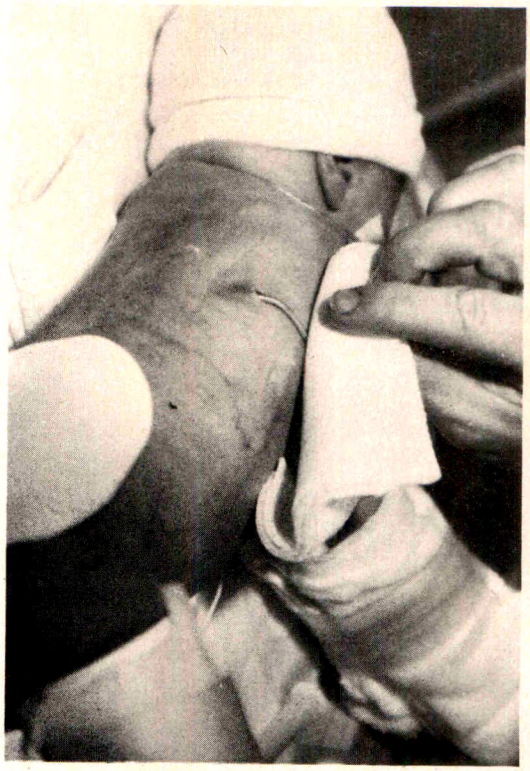


Fig. 2. At birth, shunt position is demonstrated.

port (age 14 months) the infant is showing normal growth and development without evidence of residual disease.

Comment

Fetal breathing and fluid exchange appear to be essential to normal pulmonary development; second trimester oligohydramnios may produce pulmonary hypoplasia at birth, regardless of cause.[2] Previous literature suggests that early cystic adenomatoid malformation associated with hydrops also results in insufficient pulmonary development to support life after birth and is uniformly fatal.[1] However, we have demonstrated that even with massive hydrops and extreme compression of pulmonary structures before 20 weeks' gestation, aggressive fetal surgical intervention may result in drainage of the cyst and resolution of the hydrops and allow normal pulmonary development. In addition to cystic adenomatoid malformation, conditions to be considered in the differential diagnosis of antenatally diagnosed fetal thoracic cystic masses include pulmonary sequestration, bronchiogenic and enteric cysts, and diaphragmatic hernia. Although cystic adenomatoid malformation may result in fetal or neonatal death, it is only rarely associated with other congenital anomalies. Associated fetal hydrops is felt to be the result of venacaval obstruction and cardiac compression from the extreme mediastinal shift caused by these lesions. Although it has been suggested that loss of protein from the lesion into the amniotic fluid may contribute to such hydrops, this did not appear to be the case in our patient because the fluid aspirated from the cyst was aproteinaceous.[1]

When cystic adenomatoid malformation is diagnosed antenatally, a team approach is imperative, involving a perinatologist who is skilled in real-time ultrasonography and direct ultrasound-guided intrauterine manipulation, as well as a neonatologist and pediatric surgeon. There can be no question that such an aggressive multidisciplinary approach to an anomalous fetus who classically would have been a candidate only for abortion resulted, in this case, in a normal, healthy infant. This procedure may be added to the slowly growing list of fetal surgical procedures that may now be performed on our patient, the fetus.

REFERENCES

1. Adzick NS, Harrison MR, Glick PL, et al. Fetal cystic adenomatoid malformation: pre-natal diagnosis and natural history. J Pediatr Surg 1985;20:483.
2. King JC, Mitzner W, Butterfield AB, et al. Effect of induced oligohydramnios on fetal lung development. Am J Obstet Gynecol 1986;154:823.

Labial agglutination secondary to a herpes simplex II infection

Bryan J. DeMarco, M.D., R. Scott Crandall, M.D., and Myroslaw M. Hreshchyshyn, M.D.
Buffalo, New York

A case is described of a young woman with labial agglutination secondary to a herpes simplex II infection that was successfully treated with laser vaporization. (AM J OBSTET GYNECOL 1987;157:296-7.)

Key words: Labial agglutination, herpes simplex II infection, laser vaporization

This report presents a case of labial agglutination. It is of interest because it is the first such case to be reported as a complication of a herpes simplex II infection.

Case report

A 35-year-old white nulligravid woman with normal monthly menses presented with occlusion of the introitus. She reported an outbreak of severe genital herpes simplex II infection 2½ years previously, which was confirmed by a viral culture. She described suffering from a high fever, arthralgias, and myalgias that were treated with oral analgesics during this attack. Shortly after this episode she noticed occlusion of the introitus, being unable to have intercourse or insert a tampon.

Examination of the external genitalia revealed agglutination of the labia minora in the midline with multiple small fenestrations present (Fig. 1). Anteriorly, the urethral meatus was visible. A single digit was inserted posteriorly to reveal a normal cervix and uterus.

The patient was treated surgically on an ambulatory basis with laser therapy. The adhesion band was vaporized with a Sharplan 1100 laser at a power density

From the Department of Gynecology-Obstetrics, Childrens Hospital, University at Buffalo, State University of New York.
Received for publication January 26, 1987; accepted March 3, 1987.
Reprint requests: Bryan J. DeMarco, M.D., Department of Gynecology-Obstetrics, Childrens Hospital, 140 Hodge Ave., Buffalo, NY 14222.

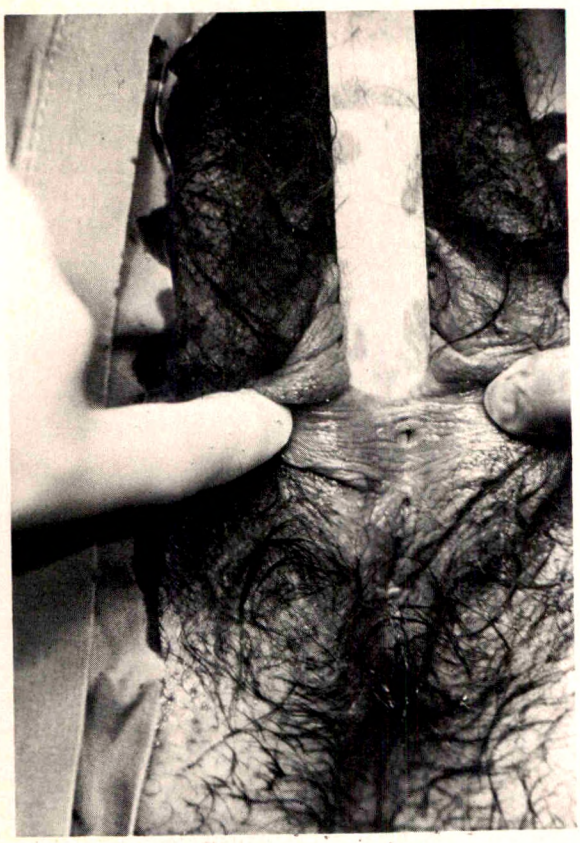


Fig. 1. Before laser therapy.



Fig. 2. After laser therapy.

of 469 W/cm² while she was under local anesthesia. Two wooden tongue depressors were placed behind the fused labia to protect the urethra and the adjacent vaginal mucosa. The labia were separated without difficulty with complete hemostasis. A gauze coated with petroleum jelly was then placed in the vaginal outlet to prevent adhesion reformation. Postoperative follow-up at 2, 6, and 8 weeks revealed no reformation of adhesions and a normal-appearing introitus (Fig. 2). The patient denied any further difficulty with intercourse.

Comment

Genital herpes has been known to predispose to many complications. There have been reported cases of urethral stricture, genital edema, and lymphatic suppuration. This is the first known, reported case of labial agglutination as a complication of genital herpes simplex II infection. Labial fusion has been previously reported in premenstrual girls and postmenopausal women. These are usually secondary to trauma, bacterial infections, or estrogen deficiency. The treatment of labial agglutination has been primarily estrogen administration for a 3- to 4-week period. This patient was treated successfully by laser vaporization while under local anesthesia.

REFERENCES

1. Caparo CJ, Greenberg H. Adhesion of labia minora: a study of 50 patients. Obstet Gynecol 1972;39:65.
2. Chow AW. Genital infections with type 2 herpes simplex virus: a common venereal disease. Postgrad Med 1975; 58:2.

Barriers to receiving adequate prenatal care

Marilyn L. Poland, Ph.D., R.N., Joel W. Ager, Ph.D., and Jane M. Olson, B.A.
Detroit, Michigan

One hundred eleven postpartum patients who received varying amounts of prenatal care (no care, inadequate care, intermediate care, and adequate care) were assessed for demographic, medical, and sociocultural factors by interview and review of the medical chart. Six sociocultural factors identified by stepwise multiple regression contributed to 49% of the variance for amount of prenatal care: amount of insurance, attitudes toward health professionals, delays in suspecting pregnancy, delay in telling others about the pregnancy, perception of the importance of prenatal care, and initial attitudes about being pregnant. Results are discussed in terms of developing outreach programs altered to the patient's needs and life-styles. (Am J Obstet Gynecol 1987;157:297-303.)

Key words: Prenatal care, barriers, outreach

Most (78%) women in this country begin prenatal care in the first trimester of pregnancy. However, 5% enter care in the third trimester or not at all.[1] The importance of receiving adequate prenatal care was highlighted recently by the U.S. Surgeon General, who set a national goal for at least 90% of pregnant women in each racial and ethnic group to begin prenatal care in the first trimester by 1990.[2]

Studies of natality statistics indicate that mothers who are single, black, under 18 years of age, and poor are most likely to receive little or no prenatal care.[3] Attitudinal surveys of women who receive inadequate prenatal care report that negative attitudes toward being pregnant, low perceived value of prenatal care, and structural barriers such as lack of insurance, transportation, and babysitters discourage prenatal visits.[4-8] Few studies examine the relative effects of demographic, medical, and attitudinal factors on amount of prenatal care received.

The following study was undertaken (1) to determine the relative effects of sociodemographic, medical, attitudinal, cultural, and structural variables on prenatal care seeking in low income, primarily black women who receive varying amounts of prenatal care and (2) to identify specific areas within the health care domain that might be altered to encourage women to receive adequate care.

From the Department of Obstetrics and Gynecology, Wayne State University School of Medicine.
Received for publication October 3, 1986; revised February 23, 1987; accepted April 27, 1987.
Reprint requests: Marilyn L. Poland, Ph.D., R.N., Associate Professor, Department of Obstetrics and Gynecology, 275 East Hancock, Detroit, MI 48201.

Material and methods

Sample. A sample of 111 women who were likely to have received varying amounts of prenatal care and

Table I. List of variables

Background variables	*Medical variables*	*Sociocultural variables*	*Content analysis*
Age	Mo. gestation prenatal care began	Mo. gestation mother suspected pregnancy	
Race	No. and sources of prenatal care	Early signs of pregnancy	
Marital status	No. of prenatal visits	Major source of transportation to prenatal care	
Parity	Risk status (antenatal score)	Other structural barriers to prenatal care	
	Length of gestation	Time delay in telling others about pregnancy	Time delay (mos): suspected pregnancy to telling second person
		Initial attitude toward pregnancy	(1) Negative (wanted abortion), (2) mixed, (3) positive
		Amount of insurance	(1) No insurance, (2) on/off insurance, (3) insurance over entire pregnancy
		Importance of prenatal care	(1) Not important, (2) somewhat important (3) very important
		Attitude toward health professionals	(1) Negative (fear, hate, bad experiences), (2) mixed, (3) positive

who delivered at Hutzel Hospital in Detroit were studied 1 to 5 days postpartum. The women were all English speaking, had been delivered of viable infants, and agreed voluntarily to participate. The research protocol was approved by the Institutional Review Board. The sample consisted of women who were listed as "walk-ins" (did not have a physician associated with the hospital) and those who received prenatal care in the hospital's antenatal clinic. The sample was collected over an 18-month period, and we attempted to interview the same number of walk-ins and clinic patients in any given week.

Data collection and scoring. Data collection consisted of a structured interview with open-ended and fixed-choice questions and review of the mother's medical chart. The interview explored experiences, attitudes, beliefs, values, and health-seeking behaviors from the time the woman thought she might be pregnant through delivery. The subject's early recollections of her pregnancy included how (symptoms) and when she first knew she was pregnant, how she felt about the pregnancy, who she told and when, and descriptions of the reactions and advice she received. Prenatal care-seeking activities included a list of all providers, times of initial visits, locations, reasons for visits, total number of visits, cost, problems obtaining care, and an assessment of value and benefit. Subjects were also asked to list important items physicians could do for them, the importance of seeing a doctor, how they obtained information about pregnancy, and describe experiences with labor and delivery.

Responses to open-ended questions were recorded in the subject's own words and then analyzed by content analysis and converted into ordinal scales. Content analysis consists of the placement of items, responses, or behaviors into predefined categories.[9] For example, amount of insurance was coded as "no insurance" if the subject had no insurance over the pregnancy period, "part insurance" if she had insurance over part of the pregnancy, and "total insurance" if she was covered throughout pregnancy. A scale from 1 to 3 represented these categories. Reliability of these scales was assessed by comparing scores of two coders who scored responses independently. Scaled responses with agreement less than 0.90 by rank order correlation were deleted from the analysis. Table I lists the major variables analyzed and organized into three categories: background variables, medical variables, and sociocultural variables. Variables derived by content analysis are also shown.

Medical chart review included obtaining sociodemographic information and selected maternal and antenatal conditions that were reliably assessed for each patient. These conditions included maternal age greater than 35 years or less than 16 years, parity greater than 5, diabetes mellitus, hypertension, heart disease, hematocrit less than 27% at delivery, drug abuse, gestation greater than 42 weeks, sexually transmitted disease, and isoimmunization. Items were given unit weights and totaled to yield a risk score.

Statistical analysis. The basic analytic problem was the identification of factors (variables) that distinguish among women receiving varying amounts of prenatal care. Amount of prenatal care was determined by the month of gestation that care began, number of appointments, and length of gestation. Kessner et al.[10] proposed a formula that accounts for these factors using the standards of the American College of Obstetricians and Gynecologists for number of prenatal care visits and yielded three levels of care: adequate, in-

Table II. Differences among groups on background and medical variables (analysis of variance)

Comparison	Groups				$p <$
	Adequate (n = 35)	*Intermediate (n = 35)*	*Inadequate (n = 19)*	*No care (n = 22)*	
Age	24.5	21.6	24.5	25.2	NS
Race—black (%)	84.6	88.2	88.6	87.2	NS
Marital status—married (%)	25.0	22.3	23.4	29.3	NS
Parity	1.2	1.0	2.1	1.7	0.05
Antenatal risk score	0.54	0.35	0.90	0.86	0.05
No. of prenatal providers	1.51	1.22	1.25	0	NS

termediate, and inadequate care. We modified this procedure to give us four levels of prenatal care: adequate (n = 35), intermediate (n = 35), inadequate (n = 19), and no care (n = 22). For example, a woman who registered in the first trimester, kept 7 monthly appointments, and delivered at 32 weeks received adequate prenatal care; a woman who registered in the sixth month, kept 7 appointments, and delivered at term received an intermediate amount of care. Women who had a prgnancy test only were placed in the no care group. For analyses requiring dichotomized groups, amount of prenatal care was categorized into appropriate care (adequate/intermediate) and poor care (inadequate/no care) groups.

The analysis was conducted in several steps. First, frequency distributions and descriptive statistics including intercorrelations were obtained for all variables within each care group. Second, variables were compared across the four care groups by one-way analysis of variance, intercorrelation, and χ^2 analyses. Variables for which complete data were available and for which there was a pattern of differences across the four groups were retained for further analysis. Third, the selected independent (predictor) variables, and the background and medical variables of age, parity, and total risk score, were entered into a stepwise multiple regression analysis to identify variables predictive of women who received varying amounts of prenatal care. In this analysis, we considered the four care groups to be equally spaced on the continuum of prenatal care.

Fourth, we entered the significant predictor variables identified by multiple regression into a stepwise, linear discriminant, function analysis using the dichotomized care groups (appropriate versus poor) as the dependent variable. In addition to identifying the significant discriminating predictors, this analysis also allowed us to determine how well these patients could be classified into the two groups on the basis of the significant predictor variables.

Fifth, using the set of significant predictors from the linear discriminant, function analysis, evaluation of risk of receiving poor care as a function of the number of "risk" factors for the woman was obtained by means of a logistic regression analysis. For this analysis, we dichotomized the predictor variables into "at risk" versus "no risk" categories. The "at risk" categories for each variable were: (1) insurance (scores 2 and 3), (2) month gestation pregnancy was suspected (2 or more months), (3) attitudes toward health professionals (scores 2 and 3), (4) initial attitude toward pregnancy (scores 2 and 3), (5) time delay in telling others (1 or more months), (6) perceived importance of prenatal care (scores 2 and 3).

Results

Comparisons among the four care groups on selected variables were made by one-way analysis of variance. Results for background and medical variables are shown in Table II.

Background and medical variables. There were no significant differences in race, age, number of prenatal providers, and marital status, although the trend was that those receiving better care were more apt to be married. All 21 adolescents less than 19 years of age received some prenatal care, although 15 registered in the second trimester. Almost 40% (35/89) of women who received any prenatal care went to more than one provider. Most (64%) prenatal care was provided by an obstetrician or family physician in a clinic or private office. However, 36% of the women made between one and 16 visits to emergency walk-in centers located throughout Detroit. These centers were popular because they were convenient (most women walked to them), no appointment was necessary (some women had no phone), and they were inexpensive (most took Medicaid or they charged an average $10/visit). On the negative side, the physicians were felt to be less competent, they provided no pregnancy literature, and no return appointments were given.

There were significant differences among groups for parity with better care received by those with lower parity. This corroborated statements made by many women across groups that prenatal care was most important for a first pregnancy, whereas women who had

Table III. Differences among groups on sociocultural variables (analysis of variance)

Comparison	Groups*				$p <$
	Adequate (n = 35)	*Intermediate (n = 35)*	*Inadequate (n = 19)*	*No care (n = 22)*	
Amount of insurance	2.7	2.7	2.3	1.5	0.001
Mo. pregnancy suspected	1.5	1.9	2.2	2.3	NS
Initial attitude toward pregnancy	2.7	2.7	2.4	2.0	0.01
Delay telling others (No. of mo)	0.5	0.7	1.6	1.2	0.01
Importance of care	2.9	2.9	2.4	2.6	0.01
Attitude toward health professionals	2.6	2.4	2.0	1.7	0.01
Major source of transportation					
Car (%)	50.0	34.5	21.9	—	
Cab (%)	11.0	7.3	9.3	—	
Bus (%)	24.7	41.8	31.3	—	NS
Walked (%)	14.3	16.4	37.5	—	

*Contrast between two higher groups versus two lower groups was significant: $t = 2.47$, degrees of freedom = 109, $p < 0.05$.

Table IV. Stepwise, linear, multiple regression of variables with amount of prenatal care (n = 110)

*Variables**	*Multiple R*	R^2	*F to remove*
Insurance	0.43	0.19	22.77
Attitude toward health professionals	0.57	0.32	8.75
Initial attitude toward pregnancy	0.61	0.37	12.75
Mo pregnancy suspected	0.66	0.43	12.67
Importance of prenatal care	0.69	0.48	7.82
Delay in telling others of pregnancy	0.70	0.49	4.03

*Variables are listed in order of entry. The variables of age, parity, maternal risk, and substance abuse failed to attain significance.

a baby felt that they already knew how to take care of themselves and needed less care. There were also significant differences among groups in the antenatal risk score. Women in the inadequate and no care groups had higher risk scores; the differences were primarily because of one factor—use of illicit drugs. Almost 31% (13/42) of women in the poor care group abused drugs, primarily heroin, compared with 7% of women in the adequate care group.

Sociocultural variables. Differences among the four groups on six sociocultural variables and the principal means of transportation for the three groups receiving care are seen in Table III. Of these seven variables, five showed significant differences in the expected directions among the groups. In all five instances, the major differences were between the higher two (appropriate care) and lower two (poor care) groups. The sixth variable, month of gestation the woman suspected she might be pregnant, was significant when the four groups were dichotomized into appropriate versus poor groups.

The largest difference among the groups was amount of insurance; the no care group had much less insurance than the other three groups. All but three women in the sample who had insurance had Medicaid; 12 had no insurance. Interestingly, only three of these 12 patients had sought Medicaid insurance, and two were told they were ineligible. The others were Medicaid eligible but did not pursue insurance for a variety of reasons, primarily related to lack of motivation and the difficulties of applying.

Although there were no significant differences among the three groups who received prenatal care on the major source of transportation used, there was a linear trend, with women receiving the most care who used the most expensive modes of transportation such as a car or a taxi, whereas women who received less care were more likely to use public transportation or walk. All women who used public transportation complained about unreliable bus schedules, poor weather, and dangerous conditions. There were also no differences among groups for early signs of pregnancy or problems seeking care such as need for a babysitter, long wait to see a doctor, or seeing different doctors. Most women across groups complained about these problems.

Table IV lists the six significant predictors from the multiple regression analysis in order of entry. Amount of insurance was the best predictor of amount of prenatal care as evaluated by the F to remove, followed by initial attitude toward pregnancy and the month in which the pregnancy was suspected. Attitudes toward health professionals and importance of prenatal care were also strong predictors. One other variable, time delay in telling others about the pregnancy, was also marginally significant. The multiple correlation for the six predictors was 0.70, accounting for about one half

Table V. Discriminant function analysis with two prenatal care groups as criterion (poor care versus appropriate care) (n = 110)*

Group	*Correct classification (%)*	*No. cases predicted*		
		Poor care	*Appropriate care*	*Totals*
No. of cases observed				
Poor care	83	33	7	40
Appropriate care	84	11	59	70
Totals	84	44	66	110

*These are jackknifed values.

Table VI. Logistic regression analysis predicting probability of receiving poor care as a function of "risk" score (n = 110)

Risk score	*No. of cases in poor care group*	*No. of cases in adequate care group*	*Observed proportion receiving poor care*	*Predicted proportion receiving poor care*
6	1	0	1.00	0.98
5	5	0	1.00	0.93
4	7	2	0.78	0.79
3	17	17	0.50	0.53
2	7	20	0.26	0.25
1	3	23	0.12	0.09
0	0	8	0.00	0.03
Total	40	70		

of the variance of amount of care. We note that the six predictors were relatively uncorrelated, with the highest correlation of −0.26 between attitudes toward health professionals and month that pregnancy was suspected.

Table V shows the jackknifed classification results from the linear discriminant, function analysis based on the six significant predictors. The overall percentage of correct classification was 84%. Sensitivity was 83%, with 33 of 44 poor care patients correctly identified. Specificity was similar (84%), with 59 of 70 of the appropriate care group correctly classified. The false-positive and false-negative rates were 11% (seven of 66) and 25% (11 of 44), respectively.

As a final analysis, we evaluated the prediction of poor care from an "at risk" score derived from the six predictor variables as previously described. The composite score for "risk" of receiving poor care ranged from 0 to 6. Table VI shows the probability or receiving poor care as a function of amount of "risk." The predicted probabilities from the model ranged from 0.98 for a risk score of 6 down to 0.03 for a risk score of 0.

Comment

The purpose of this study was to examine the relative effects of demographic, medical, and sociocultural factors on amount of prenatal care received. The key finding is that six sociocultural factors that were consistently related to amount of prenatal care were identified: amount of insurance, attitude toward health professionals, delay in suspecting pregnancy, delay in telling others about the pregnancy, perception of the importance of prenatal care, and initial attitudes about the pregnancy, which together predicted about one half of the variance for amount of prenatal care.

Before further discussion of the results, three caveats must be made. The most important concerns the issue of causality in a correlational design. We do not claim that the six variables found to predict level of care are necessarily causative. Although lack of insurance might certainly be a reason for not seeking care, it might also reflect a more pervasive attitudinal problem concerning the care for oneself. Similar comments can be made about variables of perceived importance of prenatal care and attitudes toward health professionals. The question of the causal status of the predictors is, of course, critical for both gaining a better understanding of care-seeking behaviors and designing interventions and policies that will improve the amount of care sought and received. To explore possible causal models involving these variables, we intend to conduct path analyses on a larger and more heterogeneous sample currently being collected.

A second caveat concerns the retrospective nature of the data collection. The nature of the sample, particularly the target walk-in group, imposes this constraint.

Women who receive no prenatal care are unavailable for study in an antenatal clinic.

The third caveat concerns the generalizability of the results. The present sample represented a fairly homogenous group of women who were generally poor, primarily black, and for the most part lived in inner city Detroit.

Despite these caveats, our findings indicate similarities and differences between women receiving appropriate amounts of prenatal care and those receiving poor care. They were similar in age, race, marital status, number of prenatal providers they used, major sources of transportation to prenatal care, their need for babysitters, and problems with providers, such as long waiting times and seeing different physicians. The fact that we found no differences in race or marital status could be from the homogeneity of the sample. Although there were no significant differences in age, women receiving intermediate amounts of care were slightly younger than the other three groups. This was in large part, due to adolescents who delayed telling their parents they were pregnant and who subsequently entered care in the second trimester. The similarities in the number of prenatal providers among the groups who received care reflected the growing popularity of emergency walk-in centers as a source of prenatal care for this population. Although most women who used them subsequently transferred to other providers, some received all their prenatal care there. One hypertensive mother of six kept her appointments at a perinatal center when she felt well but visited the emergency walk-in center when she did not feel well enough to take the bus and wait for long periods to see the perinatologist. The fact that there were no differences in complaints about public transportation, waiting times, and seeing different doctors indicates that this is a common problem across groups. It may be that women who have little motivation to seek prenatal care would view any problem, such as transportation, as a major barrier.

There were, however, significant differences among groups. Six factors reflecting a broad array of social supports and personal attitudes predicted about one half of the variance in amount of prenatal care received.

Social support. The two care groups differed significantly in the tangible assistance and emotional support they received during pregnancy. For example, women who received poor care had less insurance partly because they perceived great difficulty in registering for Medicaid. This included confusion over the long forms they were required to read and fill out and negative attitudes from social service workers who advised them about public assistance. The isolation they felt from social service workers extended to health care professionals, who were perceived by many women as frightening, hostile, and uncommunicative. Some women who were initially undecided about their pregnancy delayed seeking care until the second or third trimester only to encounter professionals who chastised them for registering late. The lack of emotional support and assistance was also apparent in their families and friends. Women in the poor care group delayed telling others about their pregnancy because they anticipated a negative response or they felt they had no one to tell who would be interested. They were more often without a telephone and had fewer people available who would help them with tangible factors such as transportation to prenatal care.

Women who received appropriate amounts of prenatal care found friends, family, and professionals who were supportive. These women were more often transported to prenatal care by a friend or member of their family, and they received continuous advice and emotional support throughout their pregnancies.

Personal attitudes. Women who received little or no prenatal care had more negative attitudes about being pregnant (many initially wanted an abortion), the importance of prenatal care, and health professionals. They saw a physician intermittently to be "checked" or when they did not feel well. Others felt they already knew how to care for themselves from a previous pregnancy. Some believed that as long as the fetus moved, they felt alright, and they took vitamins, routine prenatal care was unnecessary. Many had had bad experiences with physicians and nurses with a previous pregnancy or felt that prenatal care was boring.

The appropriate care group had more positive attitudes about their pregnancies, and they felt that prenatal care was important to ensure a healthy baby and mother. Although some complained about discourteous service, discontinuity of care, and long waiting times, they thought that the physicians were generally competent and concerned about their welfare. Although some encountered problems receiving prenatal care, they found ways to overcome them or discounted them as inconveniences that poor persons should expect.

These findings have important clinical implications. Our system of prenatal care is based on the premise that it is an important service designed to monitor and manage the progress of pregnancy and that all pregnant women will seek prenatal care as their maternal responsibility as long as it is available. However, although prenatal care services for the poor may be available, they may be relatively inaccessible to them because of a lack of support, reliance on public transportation systems, location of clinics for the poor, differences in values and attitudes about prenatal care among some

poor women, problems associated with reading and understanding information, fear of doctors, discontinuity of care, and discourteous service.

There are two possible approaches to improve access: change the attitudes and values of women who do not seek adequate care or alter the provision of prenatal services. The latter approach is probably more feasible, since we know more about altering health services and less about how to change attitudes. In addition, by changing service delivery to meet the identified needs of patients, our understanding of their social environment and life-styles, along with concern for their health, is communicated to them. This process may be our most effective way of altering their attitudes toward seeking prenatal care.

Toward this goal, the following steps are proposed. First, experiences and attitudes of pregnant women receiving varying amounts of prenatal care should be assessed locally. This could be done as individual interviews or as focus group discussions. Second, the information derived from the women should be used (1) to identify structural barriers within the health delivery system that can be altered, (2) to identify factors that reflect personal barriers or "social risk" for not receiving prenatal care, and (3) to develop population-specific outreach efforts for "at risk" women.

This marketing approach to providing prenatal care services to the poor could assume a variety of different forms. For example, in Detroit efforts are underway to reach women who are "at risk" for not receiving adequate prenatal care through a 24-hour telephone information and referral service, community baby showers, training of welfare mothers to act as paraprofessional outreach workers, and a variety of collaborative efforts among health and social agencies (including emergency walk-in centers) designed to simplify, coordinate, and augment services to pregnant women and infants. We are using the predictor variables from this study as an indicator of social risk status to identify pregnant women who are in the greatest need of support services. Finally, interviews with new mothers are continuing to increase the size and heterogeneity of the sample to assess the relative effects of the predictor variables on birth weight.

In summary, technologic advances in prenatal care have placed the United States in the forefront of the early identification and management of maternal-fetal abnormalities. However, some women who could benefit the most from these procedures receive little or no prenatal care for some of the reasons previously listed.

Unless measures are taken to improve access, women who cannot or will not traverse the maze of people, places, and information necessary to obtain prenatal care will continue to enter our hospitals as walk-ins. Those who obtain health care may not benefit from the most appropriate obstetric care if women at high medical risk select the convenience of walk-in centers over perinatal centers or if they receive less care with a subsequent pregnancy because our manner of providing prenatal care fails to communicate its importance.

We acknowledge Drs. Robert Sokol and Paul Giblin for critical reviews of earlier drafts of this paper and the late Krystal Brown for her sensitive interviews of postpartum mothers and devotion to this project.

REFERENCES

1. Placek P. The 1980 national natality survey and national fetal mortality survey—methods used and PHS agency participation. Public Health Rep, 1984;99:111-6.
2. United States Department of Health, Education, and Welfare. Healthy people, the surgeon general's report on health promotion and disease prevention. Bethesda, Maryland: Department of Health, Education and Welfare, public no. (PHS) 79-55071, 1979.
3. Institute of Medicine. Preventing low birthweight. Washington, DC: National Academy Press, 1987.
4. Klein L. Nonregistered obstetric patients. Am J Obstet Gynecol 1971;110:795-802.
5. Kaliszer M, Kidd M. Some factors affecting attendance at ante-natal clinics. Soc Sci Med 1981;15D:421-4.
6. Bruce SL, Petric RH, Chao S, Williams AM, Imaizuma SO. Unregistered obstetric patients: factor in perinatal losses in regionalized perinatal network. NY State J Med 1979;79:1374-7.
7. Reid ME, McIlwaine GM. Consumer opinion of a hospital antenatal clinic. Soc Sci Med 1980;14:363-8.
8. Joyce K, Diffenbacher G, Greene J, Sorokin Y. Internal and external barriers to obtaining prenatal care. Soc Work Health Care 1983;9:89-96.
9. Holsti OK. Content analysis of the social sciences and humanities. Reading, Massachusetts: Addison-Wesley Publishing Co, 1969:94-149.
10. Kessner AM, Singer J, Kalk CE, Schlesinger ER. Infant death: an analysis by maternal risk and health care. Washington, DC: National Academy of Sciences, 1973.

Red blood cell zinc protoporphyrin testing for iron-deficiency anemia in pregnancy

Ron B. Schifman, M.D., James E. Thomasson, M.D., and James M. Evers, M.D.
Tucson, Arizona

The diagnostic value of ferritin, transferrin saturation, and red blood cell zinc protoporphyrin for detecting iron depletion and predicting third-trimester anemia was studied in 87 women attending a private obstetrics clinic. A decline in ferritin and transferrin saturation and an increase in red blood cell zinc protoporphyrin levels were observed in third-trimester measurements when compared with those of the first trimester. Third-trimester anemia (hemoglobin <11.5 gm/dl or 115 gm/L) was detected in 13 (15%) women. Red blood cell zinc protoporphyrin was the only test that consistently demonstrated significantly different mean values between anemic and normal subgroups. The diagnostic sensitivity and predictive value of red blood cell zinc protoporphyrin for evaluating iron depletion and risk of anemia in pregnancy compared favorably to those of ferritin and transferrin saturation measurements. The operational simplicity and low cost of red blood cell zinc protoporphyrin measurements are additional characteristics that favor this procedure for office testing. (AM J OBSTET GYNECOL 1987;157:304-7.)

Key words: Iron-deficiency anemia, protoporphyrin, pregnancy

Iron deficiency, produced by increased physiologic requirements, is the most common cause of anemia in pregnancy.[1] Iron stores can be effectively monitored with serum ferritin or transferrin saturation measurements[2] and anemia may be prevented by oral iron supplementation.[3] Red blood cell zinc protoporphyrin measurements are also capable of identifying patients with depleted iron stores,[4] although little information is available about the diagnostic value of this test in obstetric practice.

Zinc protoporphyrin accumulates in red blood cells when heme biosynthesis is disturbed by decreased iron availability.[4] Measurement of the red blood cell zinc protoporphyrin concentration therefore provides information about both storage iron and its use.[5] Furthermore, this test is analytically more suitable than serum ferritin or transferrin saturation measurements for office testing because red blood cell zinc protoporphyrin can be quickly and inexpensively assayed from a drop of whole blood by hematofluorometry.[6] Red blood cell zinc protoporphyrin testing has been successfully used to screen children, blood donors, and other groups for iron depletion.[6-8] This study evaluated the diagnostic value of red blood cell zinc protoporphyrin testing for assessing iron stores and anemia risk in patients attending a private obstetrics clinic.

From the Department of Pathology, University of Arizona College of Medicine.
This study was supported in part by Grant BRSG S07RR05675, awarded by the Biomedical Research Support Grant Program, Division of Research Resources, National Institutes of Health.
Received for publication October 3, 1986; revised February 23, 1987; accepted March 12, 1987.
Reprint requests: Ron B. Schifman, M.D., Department of Clinical Pathology, University of Arizona College of Medicine, 1501 North Campbell, Tucson, AZ 85724.

Material and methods

Eighty-seven women attending a private obstetrics clinic were studied after providing informed consent to participate. This study was approved by the Human Subjects Committee of the Arizona Health Sciences Center and Tucson Medical Center. All women received standard care and completed term pregnancies. In addition, all were prescribed oral iron supplements (ferrous fumarate, 45 mg/day) as part of a multivitamin-mineral complex.

Venous blood samples were collected from study participants at each trimester period. Hemoglobin measurements were performed by the Coulter method. The precision (coefficient of variation) of this method is 0.6% at the level of 13.0 gm/dl (130 gm/L). Serum ferritin determinations were performed by radioimmunoassay (Leeco Diagnostic, Deerfield, Michigan), with a precision (coefficient of variation) of 7% at 35 ng/ml (35 μg/L). Ferritin values <12 ng/ml (12 μg/L) were interpreted as abnormal.

Transferrin saturation was calculated as the percentage of serum iron versus total iron binding capacity and was measured by coulometry and ion-exchange methods (ESA Ferochem 3050, Bedford, Massachusetts). This method has a precision (coefficient of variation) of 5% at the level of 100 mg/dl (17.91 μmol/L).

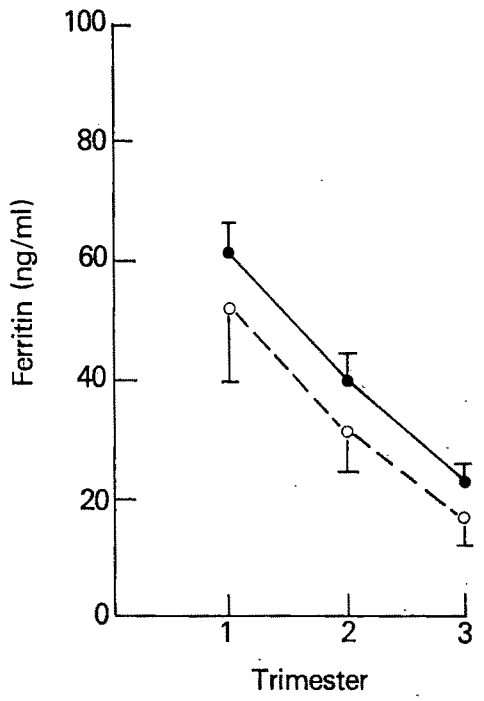


Fig. 1. Changes in ferritin during pregnancy in women developing anemia in the third trimester (○) and in women without anemia (●). Results given as mean and SEM.

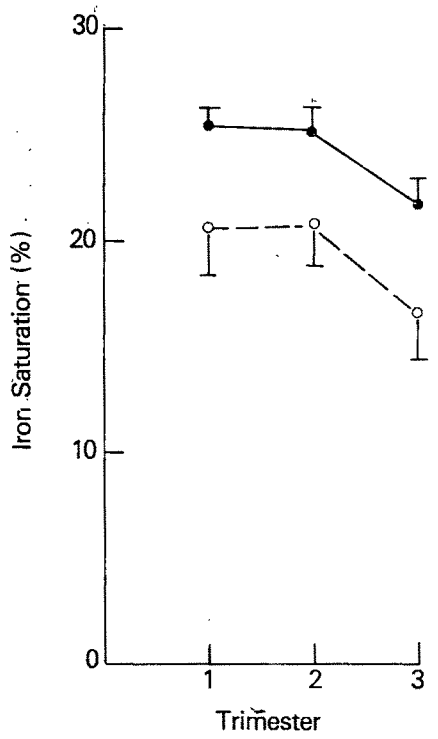


Fig. 2. Changes in iron saturation during pregnancy in women developing anemia in the third trimester (○) and in women without anemia (●). Results given as mean and SEM.

Transferrin saturation levels below 16% were interpreted as abnormal.

Red blood cell zinc protoporphyrin was measured by hematofluorometry on washed cells as previously described.[9] The hematofluorometer is a dedicated front surface fluorometer that directly measures the red blood cell zinc protoporphyrin:hemoglobin ratio as a function of the intensity of light emitted by zinc protoporphyrin fluorescing at 594 nm versus the amount of excitation light absorbed by hemoglobin at 420 nm. This method has a precision of 4% at the level of 80 μg/dl red blood cells (14.33 μmol/L red blood cells). A value of ≥60 μg/dl red blood cells (10.75 μmol/L red blood cells) was interpreted as abnormal.

All tests were performed by experienced medical technologists. The accuracy and precision of all methods were monitored with appropriate controls and external proficiency testing (College of American Pathologists and Centers for Disease Control). The Student *t* test (two tail) was used to test for statistical differences between means, and Bayes' theorem was used to calculate the sensitivity and predictive value of test results.[10] Sensitivity is defined as the percentage of true positive results to true positive plus false negative results. Predictive value of a positive (abnormal) test result is the percentage of true positive results to true positive plus false positive results.

Table I. Evaluation of test results for predicting third-trimester anemia (hemoglobin ≤ 11.5 gm/dl or 115 gm/dl)

	Trimester		
Test	*1*	*2*	*3*
Sensitivity (%)			
Red blood cell zinc protoporphyrin	38	54	62
Ferritin	23	23	54
Iron saturation	31	23	46
Predictive value of positive (abnormal) test			
Red blood cell zinc protoporphyrin	56	50	35
Ferritin	27	25	32
Iron saturation	33	15	24

Results

Thirteen (15%) of 87 women demonstrated hemoglobin concentrations equal to or below 11.5 gm/dl (115 gm/L) and seven (8%) had values below 11.0 gm/dl (110 gm/L) at the third-trimester testing period. These groups were compared with the remaining study population to evaluate the value of ferritin, transferrin saturation, and red blood cell zinc protoporphyrin for predicting third-trimester anemia (Table I). Table II

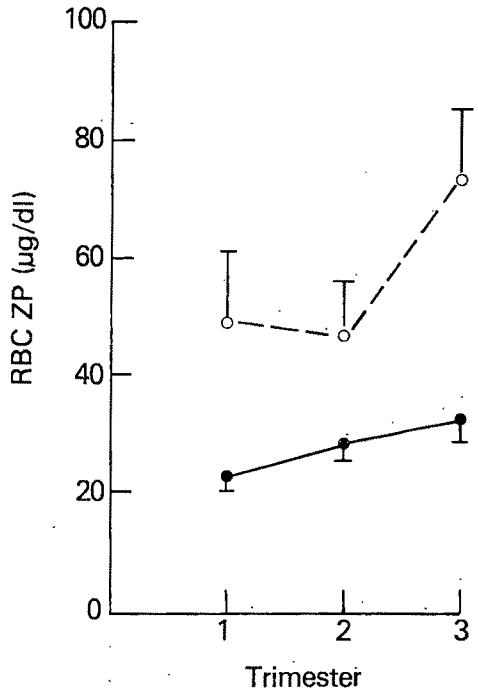


Fig. 3. Changes in red blood cell zinc protoporphyrin *(RBC ZP)* during pregnancy in women developing anemia in the third trimester (○) and in women without anemia (●). Results given as mean and SEM.

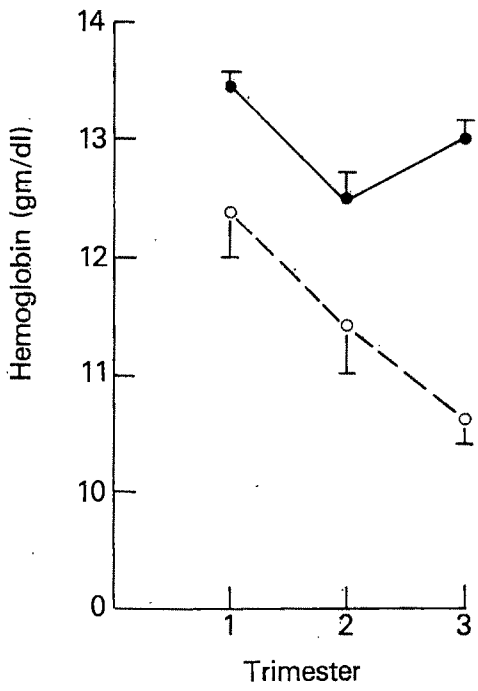


Fig. 4. Changes in hemoglobin concentration during pregnancy in women developing anemia in the third trimester (○) and in women without anemia (●). Results given as mean and SEM.

Table II. Predictive value of red blood cell zinc protoporphyrin for detecting iron depletion in pregnancy

	Trimester		
	1	*2*	*3*
Prevalence of iron depletion (%)*	22	30	46
Predictive value of negative (normal) red blood cell zinc protoporphyrin result (%)†	86	82	77
Predictive value of positive (abnormal) red blood cell zinc protoporphyrin result (%)†	44	57	70

*Interpretive criteria: Depleted iron stores defined as ferritin ≤ 12 ng/ml (12 µg/L) or iron saturation ≤ 16%.

†Abnormal red blood cell zinc protoporphyrin test defined as ≥60 µg/dl of red blood cells (10.75 µmol/L).

shows the predictive value of normal and abnormal red blood cell zinc protoporphyrin test results for assessing iron stores.

Figs. 1 through 4 show test results at each test period for the two subgroups with third-trimester hemoglobin levels above or below 11.5 gm/dl (115 gm/L). A progressive decline in ferritin concentrations was observed throughout pregnancy. However, no significant difference in mean values between anemic (n = 13) and normal (n = 74) groups was seen in the first, second, and third trimesters (t = 0.47, t = 0.84, t = 1.43, respectively; $p > 0.05$). Mean transferrin saturation results demonstrated a significant difference between subgroups only during the third trimester (t = 2.00, $p < 0.05$), while mean red blood cell zinc protoporphyrin concentrations in the anemic and normal groups were significantly different in the first (t = 3.56, $p < 0.001$), second (t = 2.78, $p < 0.01$) and third (t = 4.53, $p < 0.001$) trimesters.

Comment

The results of this study confirm previous observations that show a steady downward trend in ferritin concentrations during the first and second trimesters of pregnancy.[3] However, a further decline in iron stores, observed during the third trimester, was not expected since iron supplementation was provided to all study participants.[2, 3] This outcome may have been caused by poor compliance with prescribed iron supplements,[12] insufficient dosage of supplemental iron,[3] or impaired absorption.[13] Nevertheless, these results indicate that periodic assessment for iron-deficiency anemia is warranted during pregnancy, even in women given standard doses of iron supplements.

Third-trimester anemia was considered a harmful consequence of iron depletion and was used as an end point to assess the relative diagnostic efficiency of ferritin, transferrin saturation, and red blood cell zinc pro-

toporphyrin tests. The incidence of iron depletion steadily increased from 22% to 46% between the first and third trimesters, although only 13 (15%) women were found to have hemoglobin concentrations at or below 11.5 gm/dl (115 gm/L) during the third trimester. Holly[14] and Romslo et al.[15] observed that red blood cell protoporphyrin concentrations rose primarily during the third trimester of pregnancy in women who did not receive iron supplementation. Our study group demonstrated a gradual increase in red blood cell zinc protoporphyrin levels throughout pregnancy, with the highest levels and most marked changes observed in the third trimester in those who had anemia. This difference was not observed with ferritin or transferrin saturation results. Red blood cell zinc protoporphyrin measurements compared favorably with ferritin and transferrin saturation testing, when judged on the basis of diagnostic efficiency (that is, sensitivity and predictive value).

Iron absorption increases during pregnancy as a compensatory response to expanded utilization, and this serves to maintain normal hematopoiesis in the presence of depleted iron stores.[1] However, some women with deficient iron stores are unable to maintain adequate red blood cell mass by increasing iron absorption, and anemia may develop when apparently normal iron reserves are used ineffectively. This may explain why red blood cell zinc protoporphyrin, as a physiologic measure of iron utilization, was more effective than other tests for predicting anemia. An abnormal red blood cell zinc protoporphyrin result signifies a decompensated state of iron deficiency with ineffective heme synthesis[5] and may provide the best single choice of tests for assessing iron requirements and risk of anemia in pregnancy.

The red blood cell zinc protoporphyrin test has potential application for office testing. However, the results of this study may represent the best performance that can be achieved with the hematofluorometer, since measurements were performed on washed venous blood samples, by an experienced medical technologist. In the office laboratory, red blood cell zinc protoporphyrin will probably be measured directly from whole blood capillary samples by less skilled operators. Whole blood measurements may produce higher values because of interference by plasma constituents, and this has the potential to increase the frequency of falsely abnormal test results.[9] In addition, inaccurate results may occur from calibration failures if assay performance is not carefully monitored by appropriate control materials.[16] In spite of these potential difficulties, the hematofluorometer red blood cell zinc protoporphyrin assay has been shown to be an effective "field" method in iron-depletion screening programs.[6,7] It will be necessary to verify that reliable method performance and diagnostic effectiveness can also be maintained under typical office laboratory conditions. At the present time, the red blood cell zinc protoporphyrin test should be considered as a cost-effective alternative to other laboratory methods for identifying iron depletion and evaluating the potential for anemia in pregnancy.

REFERENCES

1. McFee JG. Iron metabolism and iron deficiency during pregnancy. Clin Obstet Gynecol 1979;22:799-808.
2. Taylor DJ. Prophylaxis and treatment of anaemia during pregnancy. Clin Obstet Gynecol 1981;8:297-314.
3. Thane-Toe T-T. The effects of oral iron supplementation on ferritin levels in pregnant Burmese women. Am J Clin Nutr 1982;35:95-9.
4. Lamola AA, Yamane T. Zinc protoporphyrin in the erythrocytes of patients with lead intoxication and iron deficiency anemia. Science 1974;186:936-8.
5. Finch CA, Huebers H. Perspectives in iron metabolism. N Engl J Med 1982;306:1520-8.
6. Schifman RB, Rivers SL, Finley PR, Thies C. RBC zinc protoporphyrin to screen blood donors for iron deficiency anemia. JAMA 1982;248:2012-5.
7. Hershko C, Bar-Or D, Gaziel E, et al. Diagnosis of iron deficiency anemia in a rural population of children: relative usefulness of serum ferritin, red cell protoporphyrin, red cell indices and transferrin saturation determinations. Am J Clin Nutr 1981;34:1600-10.
8. Yip R, Dallman PR. Developmental changes in erythrocyte protoporphyrin: roles of iron deficiency and lead toxicity. J Pediatr 1984;104:710-3.
9. Schifman RB, Finley PR. Measurement of near-normal concentrations of erythrocyte protoporphyrin with the hematofluorometer: influence of plasma on front surface illumination assay. Clin Chem 1981;27:153-6.
10. Galen RS, Gambino SR. Beyond normality: the predictive value and efficiency of medical diagnosis. New York: John Wiley, 1975.
11. Fenton V, Cavill I, Fisher J. Iron stores in pregnancy. J Haematol 1977;37:145-9.
12. Bonnar J, Goldberg A, Smith JA. Do pregnant women take their iron? Lancet 1969;1:457-8.
13. Seligman PA, Caskey JH, Frazier JL, et al. Measurement of iron absorption from prenatal multivitamin-mineral supplements. Obstet Gynecol 1983;61:356-62.
14. Holly RG. The iron and iron-binding capacity of serum and the erythrocyte protoporphyrin in pregnancy. Obstet Gynecol 1953;2:119-26.
15. Romslo I, Haram K, Sagen N, Augensen K. Iron requirement in normal pregnancy as assessed by serum ferritin, serum transferrin and erythrocyte protoporphyrin determinations. Br J Obstet Gynaecol 1983;90:101-7.
16. Balamut R, Doran D, Giridhar G, et al. Systematic error between erythrocyte protoporphyrin in proficiency test samples and patients' samples as measured with two hematofluorometers. Clin Chem 1982;28:2421-2.

The normal parturient's admission temperature

David B. Acker, M.D., Erica B. Schulman, M.A., Bernard J. Ransil, M.D., Benjamin P. Sachs, M.B., B.S., D.P.H., and Emanuel A. Friedman, M.D., Sc.D.
Boston, Massachusetts

The relationship between oral temperature taken at the time of admission and the duration of elapsed labor was evaluated. Onset of labor data revealed a diurnal distribution with an apogee at midnight to 2 AM and a nadir at 11 AM to noon. Temperature data ranged from 94.3° to 99.7° F (34.6° to 37.6° C). The mean temperature of 97.8° ± 0.8° F (36.6° ± 0.44° C) is significantly lower ($p < 0.001$) than the clinical reference temperature of 98.6° F (37.0° C). A likely explanation is the coincidental admission time (early to late morning) and the nadir of the diurnal variation in temperature. Linear regression fitted to the data (after deletion of six hypothermic outliers) yielded the relationship: temperature = 97.8° F + 0.0115 × duration of elapsed labor (temperature = 36.6° C + 0.0064 × duration of elapsed labor) for which the correlation coefficient is not statistically significant. (AM J OBSTET GYNECOL 1987;157:308-11.)

Key words: Febrile morbidity, labor, normal parturient, temperature

Fever is a sign of considerable clinical significance. It is generally defined in medicine as an elevation of body temperature above normal daily variation. Although frequently associated with infection, it may be associated with other conditions or states, including dehydration or prolonged exertion. Difficult or long labors are said to be associated with an increase (rarely above 100.4° F or 38.0° C) in the core temperature.[1,2]

A parturient's moderately elevated or slightly rising temperature presents the clinician with a diagnostic dilemma. The ability to distinguish between an early infectious process and a variation in the elevation of normal intrapartum temperature has obvious practical importance. Recent obstetric publications have not provided guidelines for interpreting this common finding.[3-5]

The purpose of this article is to provide a reliable measure of the admission temperature of a group of normal parturients and to determine the variation, if any, of temperature with respect to the duration of labor, thereby providing data to help define both the limits of normal temperature and the level at which fever can be said to exist and warrant investigation and care.

From the Charles A. Dana Research Institute and the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology and Medicine, Beth Israel Hospital and Harvard Medical School.
Supported in part by Grant RR-01032 from the General Clinical Research Centers Program of the Division of Research Resources, National Institutes of Health.
Received for publication October 22, 1986; revised March 12, 1987; accepted April 16, 1987.
Reprint requests: David B. Acker, M.D., Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Beth Israel Hospital, 330 Brookline Ave., Boston, MA 02215.

Material and methods

All patients and their singleton babies delivered at Beth Israel Hospital during the calendar year 1982, numbering 2746 pairs, were available for study. Data were abstracted from the patients' charts, collated, edited, verified, and stored on computer tapes by specially trained staff. The data base consisted of demographic and medical characteristics, obstetric complications, labor and delivery events, and neonatal outcome.

From this population, a subset was selected that fulfilled, on retrospective chart review, the following criteria: the gestational age was 37 to 41 weeks (dating from the last menstrual period); the onset of labor was spontaneous; the membranes were intact (specifically, there was no history of ruptured membranes, no evidence of amniotic fluid was noted in the vagina during the physical examination on admission, and there was subsequent documentation of either spontaneous or artificial rupture of membranes); the gravida delivered spontaneously; and there was no evidence of any antepartum, intrapartum, or postpartum infection or inflammatory disease. In all 502 patients satisfied all criteria and served as the sample population for this study.

The patient's statement concerning the onset of regular uterine contractions, as documented in the admission history, was taken as the time of onset of labor. The parturient's time of admission and concomitant vital signs, which included an oral temperature obtained with an IVAC temperature measuring system (IVAC Corp., San Diego, Calif., accuracy ± 0.2° F or 0.11° C) were recorded by the primary nurse. The difference between the two recorded times was used to define the duration of elapsed labor for the purposes of this investigation. Although additional temperature

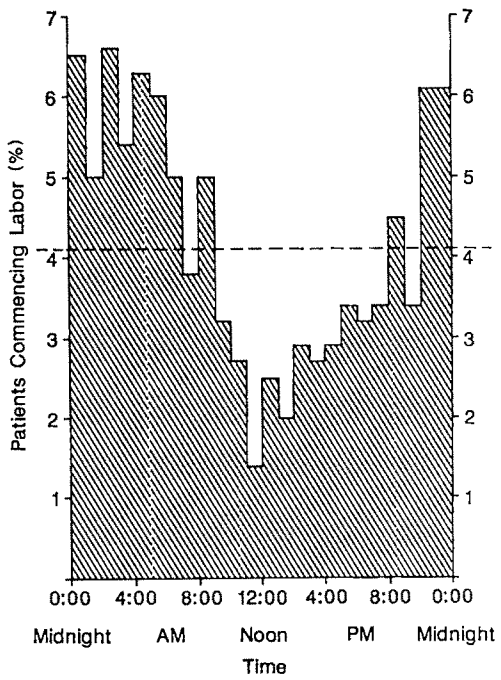


Fig. 1. Relationship between incidence of perceived initiation of spontaneous labor in the presence of intact membranes and time of day. The *dashed line* at 4.2% represents the percentage of patients who would perceive the beginning of labor if it occurred randomly.

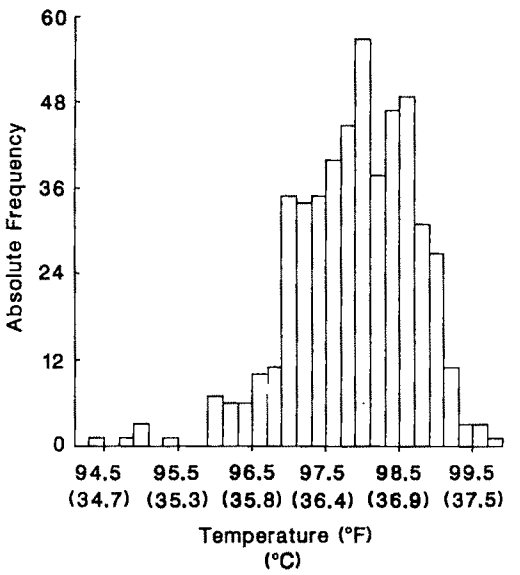


Fig. 2. Histogram (nonnormal) displays parturients' oral temperature at the time of admission to the hospital.

readings were made periodically over the course of the labor, usually at 4-hour intervals, only the first recording was used in this study.

The onset of labor, temperature on admission, and duration of elapsed labor data were evaluated for the distribution properties by means of D'Agostino's statistic, histograms, skewness (degree of asymmetry), and kurtosis (degree of peakedness) and plotted to evaluate the relationship, if any, between temperature and duration of elapsed labor. The resulting observed linear relationship was evaluated by linear least squares regression.

Results

The relationship between the percentage of gravidas perceiving the onset of regular contractions and the time of day of that perception is displayed in Fig. 1. A definite diurnal distribution is evident with an apogee between midnight to 2 AM and a nadir between 11 AM to noon.

The temperature readings obtained on admission are displayed in Fig. 2. The range for the 502 normal gravidas was 94.3° to 99.7° F (34.6° to 37.6° C). The average temperature was 97.8° ± 0.8° F (36.6° ± 0.44° C) (mean ± SD). These data were not normally distributed by D'Agostino's statistic ($p < 0.01$). A histogram showed both significant kurtosis ($p < 0.01$) and significant negative skewness ($p < 0.01$).

The duration of elapsed labor data exhibited a Poisson distribution, with a range of 0.25 to 32 hours (Fig. 3). More than half (56.4%) of the patients were admitted within 5 hours of the onset of labor. The nonnormal character of the distribution is further illustrated by the low median value, 4.25 hours, compared with an arithmetic mean of 5.89 hours, and the large coefficient of variation (84%). Distribution typing established that the duration of elapsed labor data were log normal in character with a geometric mean of 4.31 hours.

To evaluate the relationship between temperature and the duration of elapsed labor, a correlation scatterplot (Fig. 4) was made, which yielded a wedge-shaped distribution about a temperature of 97.8° F (36.6° C). A linear regression fitted to the data yielded the relationship: temperature = 97.8° + 0.0148 × duration of elapsed labor (temperature = 36.6° C + 0.0082 × duration of elapsed labor). The correlation ($r = 0.09$) and linear behavior were statistically significant at the $p = 0.04$ level.

Comment

The onset of labor data of these 502 normal parturients reveal a diurnal distribution rhythmicity that has previously been noted by Kaiser and Halberg[6] and by Shettles.[7] For a disproportionate number of patients and for reasons that remain unexplained, the initiation or the perception of the initiation of spontaneous labor

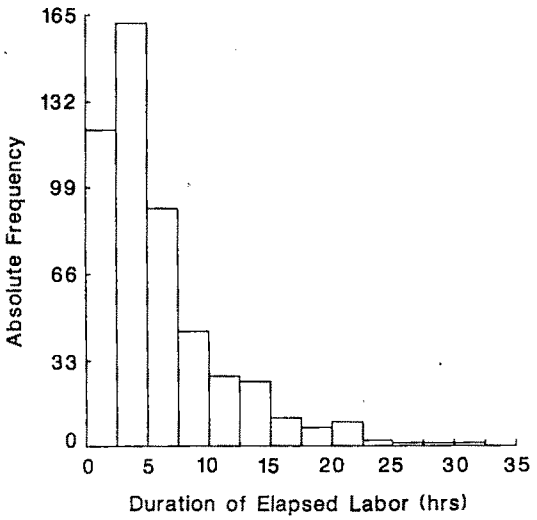


Fig. 3. Histogram displays the duration of elapsed labor at the time of admissison to the hospital.

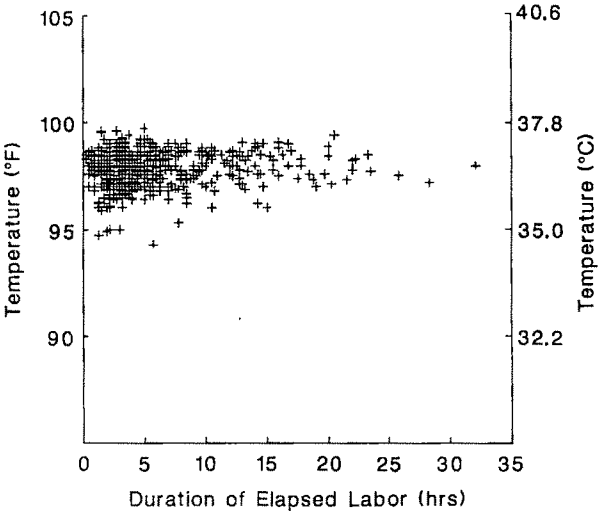


Fig. 4. Scatterplot relates the temperature at the time of admission of 502 normal parturients to the duration of elapsed labor. Note the six cases at the extreme low end of the temperature scale.

(in the presence of intact membranes) occurs during periods of rest or asleep.

The temperature data exhibit two interesting features. First, the mean temperature of 97.8° ± 0.8° F (36.6° ± 0.44° C) is significantly lower ($p < 0.001$) than the clinical reference temperature of 98.6° F (37.0° C); indeed, 79% of the patients have a temperature less than 98.6° F (37.0° C). Furthermore, the temperature distribution is skewed significantly toward lower temperatures.

The elapsed duration of labor data offers a clue to a likely explanation. Most of the patients experienced the onset of regular contractions in the very late evening or early morning hours. The median time from the onset of labor to admission was 4.25 hours. Therefore it is likely that most of the patients were admitted (and had their temperature taken) during just that period of the morning when the diurnal variation of temperature in pregnancy was at its nadir.[8]

However, several other factors might account for the findings: the accuracy of the measuring system (varies ±0.2° F or 0.11° C), an error in the clinical use of the digital thermometer, an error in the acceptance of the clinical norm of 98.6° F or 37.0° C (obtained in the days when mercury thermometers were the standard measurement device), or a bias toward hypothermia in this sample population (as suggested by the negative skewness in the histogram).

However, the inaccuracy of ±0.2° F (0.11° C) does not explain a difference of four times that value.

The digital thermometer may not be properly standardized or used, or it may detect temperature correctly, or the older mercury thermometers consistently were overread. Alternatively, both measurement devices could be in error and contribute to the relatively small difference of 0.8° F (0.44° C). These are important technologic issues that the design of this study does not address.

Turning to the remaining factor, the possibility of bias, we note that the histogram of this population (Fig. 2) shows a small group of six patients at the extreme low end of the temperature scale. The mean of this small subgroup, 94.9° ± 0.3° F (34.9° ± 0.17° C), lies 3.6 SD below the total sample mean and differs significantly from it in both mean ($p < 0.001$) and variance ($p < 0.001$). We consider the possibility that these cases represent a hypothermic subpopulation and that the parent distribution is bimodal and nonhomogeneous.

Acting on this assumption and deleting these six possible outliers yields a sample population of 496 gravidas that is normally distributed by D'Agostino's statistic and does not differ significantly in mean ± SD from the unedited sample (97.9° ± 0.7° F versus 97.8° ± 0.8° F) (36.6° ± 0.39° C versus 36.6° ± 0.44° C). Skewness ($p = 0.02$) and kurtosis ($p = 0.05$) are still statistically significant (but borderline). The range of temperatures decreases from 5.4° to 3.8° F (3.0° to 2.1° C).

A correlation scatterplot of the edited distributions (not shown) yields a linear regression of the form: temperature = 97.8° F + 0.0115 × duration of elapsed labor (temperature = 36.6° C + 0.0064 × duration of elapsed labor) for which the correlation coefficient ($r = 0.076$) is not statistically significant.

Therefore the outcome of analysis is a sample distribution of 496 individual gravidas who were admitted

with a mean temperature of 97.9° ± 0.7° F (95% confidence limits ± 0.07%) or 36.6° ± 0.39° C (95% confidence limits ± 0.04%) after a mean (arithmetic) duration of elapsed labor of 5.9 ± 5.0 hours (95% confidence limits ± 0.4%). The more precise (but less clinically familiar) geometric mean duration of elapsed labor was 4.31 hours (almost identical to the median). Therefore these cases may be regarded as a reference population from which the following clinical inferences may be drawn.

The diurnal variation in the onset of labor and a gravida's normal temperature is well established but not frequently emphasized clinical concepts. The clinician should be alert to the significance of the time of day of a gravida's admission and the resulting admission temperature.

An admission temperature of 99.4° F (37.4° C), which represents the mean + 2 SD, in a normal parturient with intact membranes is uncommon (especially in the morning hours). It can be expected to occur, with use of a one-tail value, on the basis of our distribution, once in 50 cases. Values of 100.2° F (37.9° C), which represent the mean + 3 SD, should not occur but once in 1000. The reasonableness of these frequencies can be tested against the current data base. Six of 496 patients have a temperature >99.4° F (37.4° C) whereas 10 are expected due to chance. There are no patients whose temperature exceeds 99.7° F (37.6° C), whereas two are expected due to chance.

Therefore in the absence of a history of ruptured membranes, a temperature elevation ≥99.4° F (37.4° C) may be indication of an abnormality such as unsuspected leakage of amniotic fluid (although not found in this series). A careful examination to confirm the presence of intact membranes should be initiated. This should include at the least visualization of the vaginal vault and fornices for amniotic fluid and the performance of a fern or Nitrazine paper test on suspected secretions. An ultrasonographic evaluation of amniotic fluid volume should be considered. Whether the membranes have ruptured, other sources of inflammation or infection should be considered. If the cause for the temperature elevation cannot be found, prudent management should include close observation and a well monitored in-hospital labor. Regardless of the duration of labor at the time of admission, the parturient should not be sent home.

Presently, the difference between the lower temperatures observed in this study and the more conventionally accepted reference temperature (98.6° F or 37.0° C) and the lack of any observed relationship between duration of elapsed labor and the admission temperature should not be taken to be in disagreement with the classic clinical teaching. Previous works related prolonged or difficult labors (inevitably associated with ruptured membranes and multiple pelvic examinations) to a slight rise in temperature. Our results are applicable only for the clinical conditions specified within this study. It would be inappropriate to generalize these data to other situations.

The data needed for correct interpretation of temperature in association with other than intact membranes are not available; a suitably designed prospective study is needed.

We thank Dr. Irwin H. Kaiser for his review of this manuscript and wise suggestions for its improvement. Data analysis was performed with the Core Laboratory Computer facilities.

REFERENCES

1. Eastman NJ. Williams obstetrics. 11th ed. New York: Appleton-Century-Crofts, 1960:494.
2. Greenhill JP, Friedman EA. Biological principles and modern practice of obstetrics. Philadelphia: WB Saunders Co, 1974:730.
3. Pritchard JA, MacDonald PC, Gant NF. Williams obstetrics. 17th ed. Norwalk, Conn: Appleton-Century-Crofts, 1985.
4. The American College of Obstetricians and Gynecologists. Standards for obstetric-gynecologic services. 6th ed. Washington, D.C.: 1985.
5. Hughes EC. Obstetric gynecologic terminology. Philadelphia: FA Davis Co, 1972.
6. Kaiser IH, Halberg F. Circadian periodic aspects of birth. Ann NY Acad Sci 1962;98:1056-63.
7. Shettles LB. Hourly variation in the onset of labor and rupture of membranes. Am J Obstet Gynecol 1960;79:177-9.
8. Malik J, Suk K, Brestak M, et al. Daily rhythms of leukocytes, blood pressure, pulse rate, and temperature during pregnancy. Ann NY Acad Sci 1962;98:1018-27.

Age-related changes in the female hormonal environment during reproductive life

Victoria C. Musey, M.D., Delwood C. Collins, Ph.D., Paul I. Musey, Ph.D., D. Martino-Saltzman, M.S., and John R. K. Preedy, M.D.

Atlanta and Decatur, Georgia

Previous studies have indicated that serum levels of follicle-stimulating hormone rise with age during the female reproductive life, but the effect on other hormones is not clear. We studied the effects of age, independent of pregnancy, by comparing serum hormone levels in two groups of nulliparous, premenopausal women aged 18 to 23 and 29 to 40 years. We found that increased age during reproductive life is accompanied by a significant rise in both basal and stimulated serum follicle-stimulating hormone levels. This was accompanied by an increase in the serum level of estradiol-17β and the urine levels of estradiol-17β and 17β-estradiol-17-glucosiduronate. The serum level of estrone sulfate decreased with age. Serum and urine levels of other estrogens were unchanged. The basal and stimulated levels of luteinizing hormone were also unchanged. There was a significant decrease in basal and stimulated serum prolactin levels. Serum levels of dehydroepiandrosterone and dehydroepiandrosterone sulfate decreased with age, but serum testosterone was unchanged. It is concluded that significant age-related changes in the female hormonal environment occur during the reproductive years. (Am J Obstet Gynecol 1987;157:312-7.)

Key words: Aging and hormones, estrogens, androgens, gonadotropins, prolactin

Although much information is available regarding the changes in the female hormonal environment at menarche and menopause, age-related hormonal changes during the intervening years have not been extensively investigated. Previous studies in regularly menstruating women have suggested that serum follicle-stimulating hormone (FSH) levels rise with increasing age, whereas luteinizing hormone (LH) levels remain unchanged.[1-3] However, apart from the gonadotropins, the effects of age on other hormones, including estrogens, during reproductive life are not clear.

Investigation in this area is complicated by our previous findings[4,5] that a first pregnancy causes long-term changes in the hormonal environment. Thus the effects of age and pregnancy must be separately determined. Previous studies[1-3] did not control for parity. To determine the effects of age during reproductive life, independent of pregnancy, we studied two groups of nulliparous women, a younger group aged 18 to 23 years and an older group aged 29 to 40 years. The following hormones were measured: basal and stimulated serum levels of prolactin, FSH, and LH; serum levels of estrone (E_1), estradiol-17β (E_2), estriol (E_3), and E_1 sulfate; urinary levels of total E_1, E_2 and E_3, together with their glucosiduronates were measured. The serum levels of the androgens testosterone, dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfate (DHEAS) were also measured.

From the Department of Medicine, Emory University School of Medicine, Department of Biology, Atlanta University, and the Veterans Administration Medical Center.

Supported by the National Cancer Institute Contract N01-CB-74101, National Institutes of Health Grants 5K08 AM00654 and RR00039, and the Veterans Administration. Dr. Musey is the recipient of National Institute of Arthritis, Metabolism, and Digestive Diseases Clinical Investigator Award 5 K08 AM 00654.

Received for publication August 25, 1986; revised March 5, 1987; accepted March 19, 1987.

Reprint requests: Victoria C. Musey, M.D., Department of Medicine, Emory University School of Medicine, 69 Butler St., SE, Atlanta, GA 30303.

Methods

Subjects. Thirty-five normal healthy female volunteers aged 18 to 23 years and 29 normal healthy female volunteers aged 29 to 40 years were recruited by a mass media campaign and studied after informed consent was obtained. The mean age in years ± SD was 20.4 ± 1.33 for the younger aged group and 33.9 ± 3.03 for the older aged group. All subjects were nulliparous, had a history of regular menstrual cycles, and had never taken oral contraceptives or other hormones. None were on medication at the time of the study. After medical histories were obtained and physical examinations were performed, the subjects with conditions known to be associated with abnormal hormone levels or others that would affect the study's outcome were excluded.

Experimental protocol. All subjects were studied during the early follicular phase (days 2-4) of the menstrual cycle. On day 2, each subject collected a 24-hour

urine sample. On day 3, a blood sample was collected, and a gonadotropin-releasing hormone (GnRH) stimulation test was performed. On day 4, a perphenazine-prolactin stimulation test was done (see below). Both stimulation tests were started between 8 and 9 AM after the subjects fasted overnight. All serum and urine samples were stored at −80° C until analyzed.

GnRH stimulation test. A 19-gauge butterfly needle was inserted into a forearm vein to facilitate blood collection. The needle was kept open with a heparin-saline solution. Baseline blood samples were collected for LH and FSH assays. A 100 μg bolus of GnRH was then given intravenously. Thereafter, blood samples were collected at 30-minute intervals for 90 minutes for LH and FSH assays. Serum was separated and stored as described above.

Perphenazine-prolactin stimulation test.[6] After insertion of a 19-gauge butterfly needle into a forearm vein, a baseline blood sample was collected for prolactin determination. Perphenazine (8 mg) was then administered orally, and further blood samples were collected at 3, 4, and 5 hours after the perphenazine administration to measure prolactin levels. The serum was separated and stored as above.

Procedures for hormone assays

Analysis of LH, FSH, and prolactin. Serum levels of LH, FSH, and prolactin were measured by radioimmunoassay (RIA) with commercial kits obtained from Serono Diagnostics, Inc., Braintree, Massachusetts. The reagents for measuring serum prolactin were obtained from Hybritech, San Diego, California. The intra-assay and interassay coefficients of variation established in our laboratory were LH, 2.8% and 4.4%; FSH, 3.1% and 7.7%; prolactin, 2.8% and 6.2%.

Analysis of estrogens. Serum levels of E_1, E_2, and E_3 were measured by RIA after ether extraction as previously described.[7] The intra-assay and interassay coefficients of variation for each estrogen were E_1, 6.8% and 11.2%; E_2, 5.4% and 8.2%; E_3, 6.1% and 9.1%. The E_1 sulfate level in serum was determined by direct RIA without previous hydrolysis after ether extraction to remove the free estrogens.[8] The intra-assay and interassay coefficients of variations were 6.4% and 9.5%, respectively. Urinary levels of the estrogen conjugates, E_1 glucosiduronate, E_2 17-glucosiduronate, and E_3-16α-glucosiduronate were determined by specific RIA as previously developed in our laboratory.[9] The intra-assay and interassay coefficients of variation for each compound were E_1 glucosiduronate, 6.9% and 10.8%; E_2 17-glucosiduronate, 6.5% and 10.2%; E_3 16α-glucosiduronate, 6.6% and 11.0%. The levels of E_1, E_2, and E_3 in urine were determined after hydrolysis with β-glucuronidase (*Helix pomatia*, type H-3, Sigma Diagnostics, St. Louis, Missouri). The free estrogen was extracted with ether and measured by specific RIA for the particular estrogen as previously described.[7] The coefficients of variation were the same as those of the free estrogens in serum.

Analysis of androgens. Serum levels of testosterone were measured by RIA after ether extraction as previously described.[10] The intra-assay and interassay coefficients of variation were 4.9% and 7.9%, respectively. Serum levels of DHEA were measured by RIA after ether extraction and LH-20 chromatography as previously described.[11] The intra-assay and interassay coefficients of variation were 6.4% and 10.1%, respectively. Serum levels of DHEAS were measured by RIA with a specific antiserum as previously described.[12] The intra-assay and interassay coefficients of variation were 5.4% and 8.2%, respectively. All the measurements for each hormone were done in a single assay.

Statistical analysis. Height and weight between younger and older aged groups were compared with the use of the *t* test for differences between means. A one-way analysis of variance was used to compare basal levels of the protein hormones (FSH, LH, prolactin) and the steroid hormones between younger and older aged subject groups.

A two-way repeated measures analysis of variance was used to compare stimulated levels for FSH, LH, and prolactin between groups, with main effects "younger, older" as the "between factor" and "time" (i.e., time of sampling) as the "within" or repeated measures factor.

The square root or log transformation was used to stabilize heterogeneous variances where appropriate. Subjects with missing data on the variable of interest were omitted from the analysis. Values outside the mean ± 2 SD were considered to be outliers and were also omitted. A value for $p \leq 0.05$ was taken to indicate statistical significance. Standard errors are given for descriptive purposes but are not used in the significance tests.

Results

Subjects. The mean height in inches ± SD for the younger aged group was 65.0 ± 2.39 and for the older aged group was 65.4 ± 2.31. There was no significant difference between the two means. The mean body weight expressed as percent of ideal body weight ± SD was 106.9 ± 15.6 for the younger aged group and 112.0 ± 11.4 for the older aged group. The mean body weights were not significantly different between the two groups.

Protein hormones. The basal serum FSH level was significantly higher ($p < 0.01$) in the older aged group than in the younger aged group (Table I). The mean values for stimulated FSH (Fig. 1) were also significantly higher in the older aged group ($p < 0.05$). There was no significant difference between the groups in

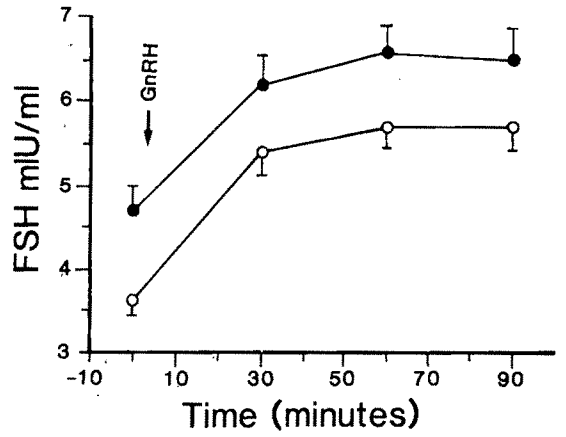


Fig. 1. Basal (time zero) and stimulated (30, 60, 90 minutes) levels of serum FSH after the intravenous administration of GnRH (100 μg) to subjects in the younger *(open circles)* and older *(closed circles)* aged groups. Both basal and stimulated values in the older aged group were significantly higher than in the younger aged group ($p < 0.01$).

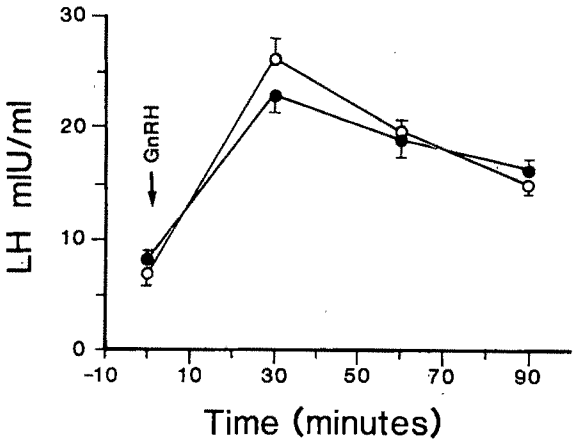


Fig. 2. Basal (time zero) and stimulated (30, 60, 90 minutes) levels of serum LH after the intravenous administration of GnRH (100 μg) to subjects in the younger *(open circles)* and older *(closed circles)* aged groups. There was no significant difference between the two groups.

either the basal serum LH or in stimulated serum LH values (Fig. 2). There was a significant ($p < 0.01$) decrease in the basal serum prolactin level in the older aged group. The stimulated prolactin values (Fig. 3) were also significantly lower ($p < 0.01$) in the older aged group.

Estrogens. The mean serum levels of E_2, E_1 sulfate, E_1, and E_3 for both groups are shown in Table II. The serum E_2 level was significantly higher ($p < 0.01$) in the older aged group, but the serum level of E_1 sulfate was significantly lower ($p < 0.01$). The serum E_1 level was lower in the older aged group, but the difference between the groups was not statistically significant. Serum E_3 levels were similar in both groups.

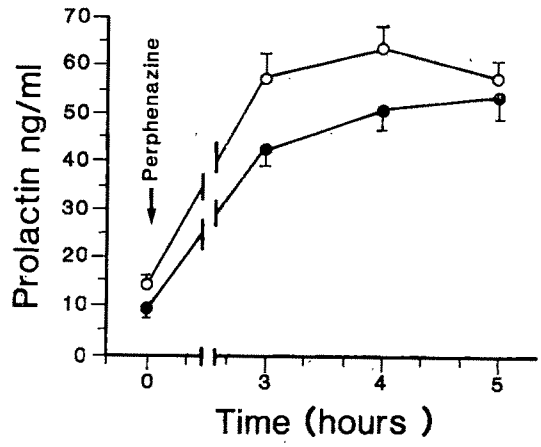


Fig. 3. Basal (time zero) and stimulated (3, 4, 5 hours) levels of serum prolactin after the oral administration of 8 mg perphenazine to subjects in the younger *(open circles)* and in the older *(closed circles)* aged groups. Both basal and stimulated values in the older aged group were significantly lower than in the younger aged group ($p < 0.01$).

The mean levels of E_2, E_1, E_3, and E_2 17-glucosiduronate in urine are shown in Table III. There were significant differences in urine levels of E_2 and E_2 17-glucosiduronate between the groups, with the older aged group having higher levels than the younger aged group. There was no significant difference between the groups in levels of E_1, E_3, E_1 glucosiduronate, and E_3 16α-glucosiduronate in urine.

Androgens. Mean serum levels of testosterone, DHEA, and DHEAS are shown in Table IV. There was no significant difference between the groups for serum testosterone. However, serum DHEA and DHEAS levels were significantly lower ($p < 0.01$) in the older aged group.

Comment

This study was designed to demonstrate the effects of age on the hormonal environment in women of reproductive age. Apart from cyclic alterations of the menstrual cycle, a first pregnancy induces long-term changes in the hormonal environment.[4, 5] Thus the effects of age and pregnancy must be separately determined. In this study we have excluded effects of pregnancy that would confound the issue by using nulliparous subjects only.

A second confounding variable is differences in serum hormone levels caused by circadian rhythm and/or episodic or pulsatile secretion. Such variation could complicate the interpretation of serum hormone values from single blood samples. In this study the time

Table I. Mean basal serum levels of FSH, LH, and prolactin in the younger and the older aged groups of nulliparous women

	Younger women (aged 18-23 yr)	*Older women (aged 29-40 yr)*	*Difference*	*p*
FSH (mIU/ml)				
Mean	3.60	4.69	+1.09	<0.01
SE	0.22	0.42		
n	35	28		
LH (mIU/ml)				
Mean	7.20	8.09	+0.89	NS
SE	0.66	0.87		
n	35	28		
Prolactin (ng/ml)				
Mean	14.2	9.36	4.84	<0.01
SE	1.87	0.88		
n	31	28		

n = Number of subjects; difference = the difference between the two means; p = the probability that the difference between means could occur by chance; NS = not significant. SE given for descriptive purposes only; it is not used in the tests for significance.

Table II. Mean serum levels (picograms per milliliter) of E_2, E_1 sulfate, E_1, and E_3 in the younger and older aged groups of nulliparous women

	Younger women (aged 18-23 yr)	*Older women (aged 29-40 yr)*	*Difference*	*p*
E_2				
Mean	61.1	74.1	+13.0	<0.01
SE	4.3	5.3		
n	34	28		
E_1 sulfate				
Mean	1043	851	−192	<0.01
SE	75	91		
n	35	27		
E_1				
Mean	72.2	62.2	−10.0	0.09
SE	5.1	5.5		
n	32	27		
E_3				
Mean	13.4	14.7	+1.30	NS
SE	0.73	0.95		
n	34	26		

See Table I for definitions.

of day at which samples were collected was controlled by obtaining all samples for basal hormone measurements in the morning (between 8 and 9 AM), thereby minimizing the effects of circadian changes. In addition, the GnRH and perphenazine stimulation tests that were used are independent of pulsatile secretion and circadian changes.

A third variable that could impact on the hormonal environment is body weight. However, this variable was discounted by finding no significant difference between the two groups of women.

Previous studies of the effects of age on serum FSH and LH levels have dealt with only basal levels. Our results in relation to basal FSH and LH levels are consistent with those reports[1-3] that suggest a significant increase in basal FSH but no change in LH. However, by using only nulliparous women in this study, we have shown that pregnancy could not have been a factor in these effects.

The GnRH stimulation test used in this study also offers additional information about age-related changes, namely, an estimate of the pituitary secretory capacity of FSH and LH. We found an increased response of FSH to GnRH stimulation. No significant change in the response of LH to GnRH stimulation was seen in the older aged group. Therefore our results suggest that both basal serum levels and pituitary secretory capacity for FSH are affected by the aging process, whereas those of LH are not affected. The mechanism for the divergent basal levels and the response

Table III. Mean levels (micrograms per 24 hours) of total E_2, total E_1, total E_3, and E_2 17-glucosiduronate in the urine of the younger and older aged groups of nulliparous women

	Younger women (aged 18-23 yr)	*Older women (aged 29-40 yr)*	*Difference*	*p*
Total E_2				
Mean	3.93	5.17	+1.24	<0.01
SE	0.27	0.45		
n	35	29		
Total E_1				
Mean	6.97	7.85	+0.88	NS
SE	0.68	0.86		
n	35	28		
Total E_3				
Mean	16.9	22.5	+5.60	NS
SE	2.22	2.87		
n	34	28		
E_2 17-glucosiduronate				
Mean	1.69	1.97	+0.28	0.03
SE	.125	0.17		
n	35	28		

See Table I for definitions.

Table IV. Mean serum levels of testosterone, DHEA, and DHEAS in younger and older aged groups of nulliparous women

	Younger women (aged 18-23 yr)	*Older women (aged 29-40 yr)*	*Difference*	*p*
Testosterone (pg/ml)				
Mean	318	303	−15	NS
SE	32	28		
n	35	27		
DHEA (ng/ml)				
Mean	4.95	3.31	−1.64	<0.01
SE	.50	.35		
n	32	27		
DHEAS (μg/dl)				
Mean	290.5	195.5	−95	<0.01
SE	25.1	21.9		
n	35	27		

See Table I for definitions.

of FSH and LH is not known but might be explained by feedback regulation of gonadotropin secretion by estrogens and/or inhibin. In this study the concomitant rise in serum levels of both FSH and E_2 argues against feedback regulation by estradiol as being responsible for the increase in FSH. Conversely, inhibin preferentially inhibits FSH secretion. Therefore decreased feedback inhibition of FSH secretion by ovarian inhibin could explain the selective rise in the FSH level.

A most interesting finding in this study was the significant age-related increase in the serum level of E_2. The increase in E_2 occurred in both the serum and urine and is consistent with one previous report.[13] The increased serum E_2 level may be caused by either increased ovarian secretion or decreased metabolism of E_2, which is metabolized principally through hepatic conjugation and E_1 formation. In this study the urine levels of both total E_2 and E_2 17-glucosiduronate were increased, whereas serum levels of E_1 sulfate and E_1 were decreased, suggesting that decreased metabolism through the E_1 pathway may account for the increased levels of E_2. However, an increase in ovarian secretion of E_2 remains a possibility that cannot be excluded with our results.

This age-related increase in serum E_2 levels in nulliparous women has important implications as a risk factor for breast cancer. It is well known that nulliparity increases the risk of breast cancer.[14, 15] Bernstein et al.[16] have recently reported that nulliparous women have higher serum levels of E_2 than parous women, indicating that previous pregnancy lowers serum levels of E_2. One possible mechanism for lowering serum E_2 levels in parous women is through the prevention of the age-related rise in the serum levels of E_2, which occurs in nulliparous women. An early first pregnancy could then reduce serum E_2 levels at an earlier age than a

late first pregnancy. This is consistent with the established finding that an early first pregnancy protects against breast cancer, whereas a late first pregnancy does not.[14, 15]

A decrease in basal serum prolactin levels with increasing age has been reported in the female subject, but postmenopausal women were included, and the long-term effects of pregnancy were not taken into account.[17-19] Yu et al.[13] found no significant change in basal serum prolactin levels with age in nulliparous women. Our results clearly indicate that there is an age-related decrease in both the basal levels of prolactin and the response of prolactin to perphenazine during the reproductive years.

We have previously reported that a first pregnancy induces long-term reduction in the secretion of prolactin.[4] In this study the effects of pregnancy were excluded by using only nulliparous women. Thus both the aging process and previous pregnancy affect prolactin secretion. However, serum prolactin levels are higher in nulliparous women than in parous women,[4, 13] indicating that a pregnancy at an early age will lower serum prolactin levels at an earlier age and for a longer period than the aging process.

There was again a divergence with regard to the androgens. There was no age-related change in serum levels of testosterone, whereas serum levels of both DHEA and DHEAS decreased. Serum DHEA and DHEAS levels decrease during pregnancy.[20] We have shown that the reduction in DHEA and DHEAS levels induced by a first pregnancy persists for several years.[5] The effects of age and pregnancy are frequently concurrent and difficult to separate. However, the effects of pregnancy were discounted in this study by using only nulliparous women. Our results clearly show that serum levels of DHEA and DHEAS change in response to age, as well as to a first pregnancy.

In summary, our studies indicate that increasing age during reproductive life in the woman is accompanied by a significant change in the hormonal environment that is independent of previous pregnancy. There is an increase in both basal serum FSH levels and the response of FSH to GnRH stimulation. In turn, this is associated with an increase in serum levels of E_2, urinary levels of total E_2 and E_2 17-glucosiduronate, and a decrease in serum levels of E_1 sulfate. Other serum and urinary estrogen levels are unchanged. Basal and GnRH stimulated levels of LH are also unchanged. Serum levels of DHEAS and DHEA decrease markedly, but testosterone remains unchanged. Both basal and perphenazine-stimulated serum levels of prolactin are markedly reduced. These changes in the hormonal milieu during the reproductive years have important implications about physiologic and pathologic conditions that are affected by reproductive hormones.

REFERENCES

1. Sherman BM, Korenman SG. Variation of the human menstrual cycle throughout reproductive life. J Clin Invest 1975;55:699-706.
2. Sherman BM, West JH, Korenman SG. The menopausal transition: analysis of LH, FSH, estradiol, and progesterone concentrations during menstrual cycles of older women. J Clin Endocrinol Metab 1976;42:629-36.
3. Reyes FI, Winter JSD, Faiman C. Pituitary-ovarian relationships preceding the menopause. I. A cross-sectional study of serum follicle-stimulating hormone, luteinizing hormone, prolactin, estradiol, and progesterone levels. Am J Obstet Gynecol 1977;129:557-64.
4. Musey VC, Collins DC, Musey PI, Martino-Saltzman D, Preedy JRK. Long-term effect of a first pregnancy on the secretion of prolactin. N Engl J Med 1987;316:229-34.
5. Musey VC, Collins DC, Brogan DR, et al. Long-term effects of a first pregnancy on the hormonal environment: estrogens and androgens. J Clin Endocrinol Metab 1987; 64:111-8.
6. Musey VC, Preedy JRK, Musey PI, et al. Prolactin and blood pressure responses to perphenazine in human subjects: comparison of the oral and intramuscular routes. Am J Med Sci 1986;291:380-5.
7. Wright K, Collins DC, Preedy JRK. The use of specific radioimmunoassays to determine the renal clearance rates of estrone and 17β-estradiol during the menstrual cycle. J Clin Endocrinol Metab 1978;47:1084-91.
8. Wright K, Collins DC, Musey PI, Preedy JRK. A specific radioimmunoassay for estrone sulfate in plasma and urine without hydrolysis. J Clin Endocrinol Metab 1978;47; 1092-8.
9. Wright K, Collins DC, Preedy JRK. Urinary excretion of estrone glucosiduronate, 17β-estradiol-17 glucosiduronate and estriol-16α glucosiduronate. Significance of proportionate differences during the menstrual cycle. Steroids 1979;34:445-56.
10. Perachio AA, Alexander M, Marr LD, Collins DC. Diurnal variations of serum testosterone levels in intact and gonadectomised female rhesus monkeys. Steroids 1977;29: 21-30.
11. Buster JE, Abraham GE. Radioimmunoassay of plasma dehydroisoandrosterone. Anal Letters 1972;5:203-7.
12. Buster JE, Abraham GE. Radioimmunoassay of plasma dehydroisoandrosterone sulfate. Anal Letters 1972;5: 543-7.
13. Yu MC, Gerkins VR, Henderson BE, Brown JB, Pike MC. Elevated levels of prolactin in nulliparous women. Br J Cancer 1981;43:826-31.
14. MacMahon B, Cole P, Brown J. Etiology of human breast cancer: a review. JNCI 1973;50:21-42.
15. Thomas DB. Do hormones cause breast cancer? Cancer 1984;53:595-604.
16. Bernstein L, Pike MC, Ross RK, Judd HL, Brown JB, Henderson BE. Estrogen and sex hormone-binding globulin levels in nulliparous and parous women. JNCI 1985;74:741-5.
17. Bulbrook RD, Wang DY, Hayward JL, Kwa HG, Cleton F. Plasma prolactin levels and age in a female population: relation to breast cancer. Int J Cancer 1981;28: 43-5.
18. Vekemans M, Robyn C. Influence of age on serum prolactin levels in women and men. Br Med J 1975;4:738-9.
19. Rose DP, Pruitt BT. Plasma prolactin levels in patients with breast cancer. Cancer 1981;48:2687-91.
20. Milewicz L, Gomez-Sanchez C, Madden JD, et al. Dehydroepiandrosterone sulfate in peripheral blood of premenopausal, pregnant and post-menopausal women and men. J Steroid Biochem 1978;9:1159-64.

Evidence that estrogen may be a key factor in hyperprolactinemic anovulation: A case report

Hideki Sakamoto, M.D., Konbai Den, M.D., Yasumasa Kondo, M.D., Katsuo Tsubata, M.D., and Shigeo Takagi, M.D.

Tokyo, Japan

Although anovulation associated with hyperprolactinemia is not an uncommon cause of infertility, the precise mechanism of the pathogenic process that induces hyperactivity (hypertrophy with hyperplasia) of pituitary lactotropes is unknown. We have recently experienced a case of anovulation and hyperprolactinemia in a woman with ergot alkaloid intolerance in whom ovulation was restored by tamoxifen citrate administration. Since tamoxifen citrate administration also suppressed prolactin levels, it was suggested that a low but sustained serum level of estradiol and consequently continuous estrogenic stimulation may be an important causative factor in the development of hyperprolactinemic anovulation. (AM J OBSTET GYNECOL 1987;157:318-9.)

Key words: Estrogen, hyperprolactinemia, tamoxifen citrate

We present here a case of hyperprolactinemia associated with infertility. Ovulation was restored in this patient with a regimen of tamoxifen citrate administration.

Case report

A 24-year-old nulligravid housewife was referred to our clinic on September 20, 1986, because of amenorrhea of 2 months' duration. She had a negative urinary human chorionic gonadotropin test and withdrew to progesterone challenge at another clinic. The serum luteinizing hormone, follicle-stimulating hormone, and estradiol levels were 26 mIU/ml, 8.5 mIU/ml, and 57.4 pg/ml, respectively. There was a moderate rise of the serum prolactin level to 110 ng/ml (upper limit of prolactin in our clinic is 30 ng/ml) and normal thyroid-stimulating hormone levels of 1.3 uU/ml.

A series of sellar x-ray studies was negative. A course of bromocriptine (5 mg/day) was initiated on September 25 but was discontinued on that day because she developed marked nausea, vomiting, and epigastrial cramps after receiving 2.5 mg of the medication. At her second visit, an antiestrogen regimen with tamoxifen citrate (20 mg/day) was started.

On the thirteenth day of the regimen, the basal body temperature rose with increased cervical mucus production. Serum estradiol, progesterone, and prolactin levels were 440 pg/ml, 0.9 ng/ml, and 78.5 ng/ml, respectively, and the tamoxifen citrate regimen was stopped on the fourteenth day (Fig. 1). The basal body temperature remained high and progesterone and prolactin levels on October 29 were 1.2 and 68.1 ng/ml. Menstruation began on November 4.

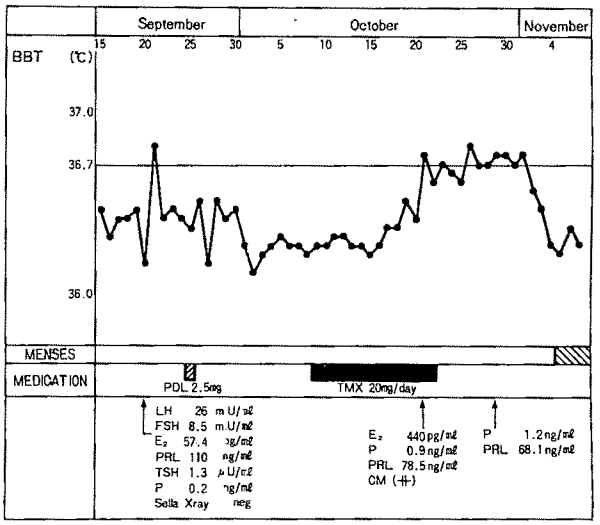


Fig. 1. Graph showing basal body temperature, menses, and effects of medication on hormone levels.

Comment

In rats, it was reported that continuous exposure to estradiol causes decreased tyrosine hydroxylase activity in the hypothalamus and a fall in dopamine levels. These changes, however, appeared to be reversible, with the catecholamine production restored and prolactin levels normalized[1] if estradiol was removed. The effect of tamoxifen citrate in the present study therefore may well be due to the blockade of estradiol action

From the Department of Obstetrics and Gynecology, Nihon University School of Medicine.

Received for publication December 3, 1986; accepted December 31, 1986.

Reprint requests: Hideki Sakamoto, M.D., Department of Obstetrics and Gynecology, Nihon University School of Medicine, 30-1, Oyaguchi, Kamimachi, Itabashi, Tokyo, Japan 173.

at the hypothalamic level and a recovery of dopaminergic function, although a direct effect of tamoxifen citrate may have modified the secretory function of the lactotropes to decrease prolactin, which in turn reestablished normal pulsatile luteinizing hormone–releasing hormone secretion from the higher neuronal center.[2]

To our best knowledge, this is the first report of successful induction of ovulation in a hyperprolactinemic women with the use of tamoxifen citrate alone. Recently one study appeared in which patients with pituitary microadenoma were successfully treated with bromocriptine and tamoxifen citrate but treatment was required for a period of several months.[3] Since our patient had only 2 months of amenorrhea with moderately elevated prolactin levels and responded to tamoxifen citrate alone, it was suggested that the early pathogenic process of the disease might at least be related to chronic estradiol output from the ovaries. In fact estradiol levels in the present case (57.4 pg/ml) are compatible with those in the midfollicular phase and not considered "hypoestrogenic."

A shorter tamoxifen citrate regimen may lessen subsequent low ovarian progesterone output (luteal defect) and improve its use in a clinical practice.

REFERENCES

1. Sakamoto H, Leranth C, MacLusky NJ, Hurlbart C, Naftolin F. Estrogen inhibition on the rat brain dopamine system is area specific and reversible [Abstract No. 140-7]. In: Proceedings of the Society for Neuroscience, Anaheim, California, 1984.
2. Cohen-Becker IR, Selmanoff M, Wise P. Hyperprolactinemia alters frequency and amplitude of pulsatile luteinizing hormone secretion in the ovariectomized rat. Neuroendocrinology 1986;42:328-33.
3. Koizumi K, Aono T. Pregnancy after combined treatment with bromocriptine and tamoxifen in two patients with pituitary prolactinomas. Fertil Steril 1986;46:312-4.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1987 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 156 (January-June) and Vol. 157 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Modulating actions of estradiol on gonadotropin-releasing hormone–stimulated prolactin secretion in postmenopausal individuals

Elisabeth Christiansen, M.D., Johannes D. Veldhuis, M.D., Alan D. Rogol, M.D., Ph.D., Paul Stumpf, M.D., and William S. Evans, M.D.

Charlottesville, Virginia, and Neptune, New Jersey

The temporal aspects of estrogen treatment on serum prolactin concentrations basally and in response to a 10 μg intravenous injection of gonadotropin-releasing hormone were assessed in eight postmenopausal women. The response of prolactin to gonadotropin-releasing hormone stimulation was compared with that of follicle-stimulating hormone and luteinizing hormone obtained simultaneously. Basal serum prolactin increased significantly ($p < 0.001$) in response to estrogen treatment, and a positive correlation was found between the serum concentrations of estradiol and prolactin ($r^2 = 0.266$; $p = 0.0011$). Gonadotropin-releasing hormone induced a significant increase in serum prolactin concentrations, which was greater after 5 to 10 days of estrogen treatment compared with that in the estrogen-depleted state ($p = 0.031$). No correlation was found between gonadotropin-releasing hormone–stimulated prolactin and luteinizing hormone or follicle-stimulating hormone release. These data demonstrate that estrogen treatment of previously hypoestrogenemic postmenopausal women potentiates gonadotropin-releasing hormone–stimulated prolactin release. Furthermore, our data suggest that the previously described paracrine interaction between the gonadotropens and lactotropes exerted by gonadotropin-releasing hormone does not appear to be mediated via increased gonadotropin release. (Am J Obstet Gynecol 1987;157:320-5.)

Key words: Prolactin, estradiol, gonadotropin-releasing hormone

Several lines of evidence suggest a strong relationship between prolactin secretion and the ambient concentration of estrogen. During the menstrual cycle, prolactin concentrations supposedly enhance coincident with relatively high circulating estrogen levels, such as just before the preovulatory luteinizing hormone (LH) surge[1] and in the middle of the luteal phase.[2] In men[3,4] and in postmenopausal women[5-7] increased basal circulating prolactin concentrations have been measured during estrogen treatment and appear to be dose related.[6] Moreover, recent studies in postmenopausal women and women with normal cycles[8-10] and in men[4] have defined enhanced prolactin secretion after gonadotropin-releasing hormone (GnRH) stimulation. However, the relationship between estrogen and GnRH-stimulated prolactin secretion remains controversial. Indeed, estrogens have been suggested to decrease,[10] increase,[4] or have no effect[8] on GnRH-stimulated prolactin secretion. Although the mechanism by which GnRH influences prolactin release is unknown, evidence has recently been presented to suggest a paracrine action of the gonadotropes on the lactotropes,[11] perhaps with follicle-stimulated hormone (FSH)–enriched gonadotropes mediating this process.[10]

We recently reported the effects of estradiol 17β, administered to postmenopausal women via a Silastic intravaginal ring on both circulating sex steroid concentrations[12] and on the stimulation of LH by GnRH.[13] We have now extended the study to (1) evaluate the temporal aspects of estrogen treatment on basal and GnRH-stimulated prolactin secretion in previously hypoestrogenic postmenopausal individuals and (2) examine the relationship between GnRH-stimulated gonadotropin and prolactin release in hypoestrogenic and estrogen-treated postmenopausal

From the Departments of Internal Medicine, Pediatrics, and Pharmacology, University of Virginia School of Medicine, and the Department of Obstetrics and Gynecology, Jersey Shore Medical Center.

Supported by National Institutes of Health RCDA 1K04 HD00634 and University of Virginia Computer Services Grant (J. D. V.); National Institutes of Health RCDA 1K04 HD00711 and University of Virginia Computer Services Grant (W. S. E.); and United States Public Health Service General Clinical Research Grant RR-847 and the CLINFO Laboratory. Dr. Christiansen is a visiting Research Fellow from Denmark.

Received for publication January 13, 1987; revised April 6, 1987; accepted April 24, 1987.

Reprint requests: William S. Evans, M.D., Department of Internal Medicine, Box 511, University of Virginia Medical Center, Charlottesville, VA 22908.

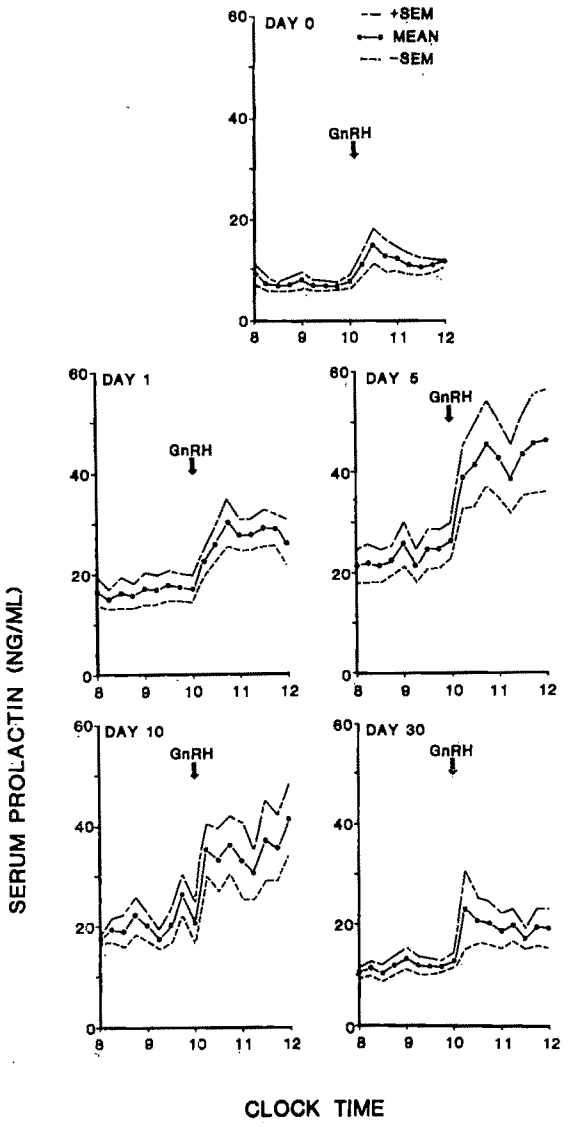


Fig. 1. Temporal profiles of serum prolactin concentrations (*ng/ml*; ±SEM) in postmenopausal women sampled at 15-minute intervals for 4 hours before estrogen treatment and on days 1, 5, 10, and 30 after intravaginal placement of a Silastic ring that contained 400 mg pure estradiol 17β. The first 2 hours of blood sampling represents basal conditions (8:00 to 10:00). A 10 μg intravenous bolus injection of GnRH was administered at 10:00 on each study day.

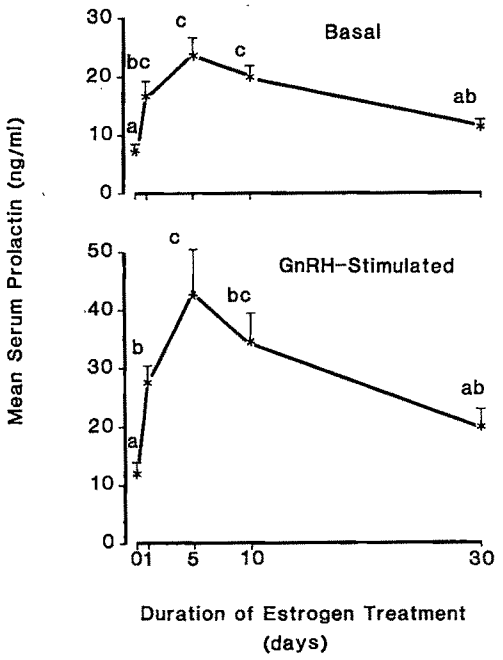


Fig. 2. Mean serum concentrations of prolactin before (day 0) and on days 1, 5, 10, and 30 of estradiol administration via an intravaginal Silastic ring. The 2-hour mean serum prolactin concentrations *(ng/ml)* are depicted for baseline conditions (top panel) and after GnRH stimulation (bottom panel). Differing labels *(a, b, c)* denote significant differences within each graph by analysis of variance ($p < 0.05$).

women. To this end, serial serum concentrations of prolactin and gonadotropins were determined basally and after a bolus injection of GnRH. Studies were performed before and at intervals (1, 5, 10, and 30 days) during the administration of estrogen.

Material and methods

Subjects. Eight healthy postmenopausal women aged 55 to 63 years (median 58 years) participated in the study, which was an extension of previously published work.[13] No subject had been treated with estrogen for at least 5 weeks before the study. Each subject had normal hepatic, renal, and hematologic function, normal thyroid hormone levels, and gonadotropin concentrations in the postmenopausal range. Written informed consent, approved by the Human Investigation Committee at the University of Virginia, was obtained before initiation of these studies.

Methods. Subjects were admitted to the Clinical Research Center at the University of Virginia on five occasions. During each admission blood samples were withdrawn at 15-minute intervals for 4 hours from 8:00 AM to 12:00 noon, through an indwelling heparin-lock cannula that was inserted 1 hour before initiation of blood sampling. An intravenous bolus injection of 10 μg GnRH (Factrel) was given at 10:00. Studies were undertaken basally (before estrogen treatment) and on days 1, 5, 10, and 30 of estradiol 17β replacement delivered via an intravaginal Silastic ring that contained 400 mg of estradiol 17β.[12-14]

Assays. All blood samples were assayed for prolactin in duplicate by radioimmunoassay as previously described.[15] Intraassay and interassay coefficients of variation were 8.3% and 8.9% at 6.6 ng/ml and 8.4% and 14.5% at 76.1 ng/ml. LH and FSH concentrations in

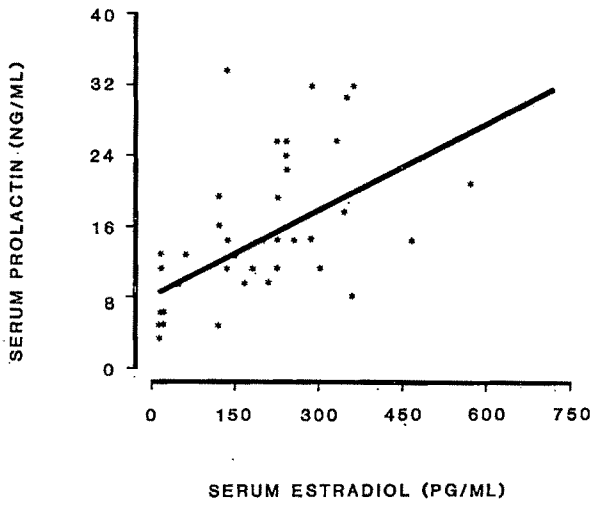


Fig. 3. Mean baseline serum prolactin was significantly and positively correlated ($r^2 = 0.266$; $p = 0.0011$) with the circulating level of estradiol obtained in postmenopausal women before and during estradiol replacement.

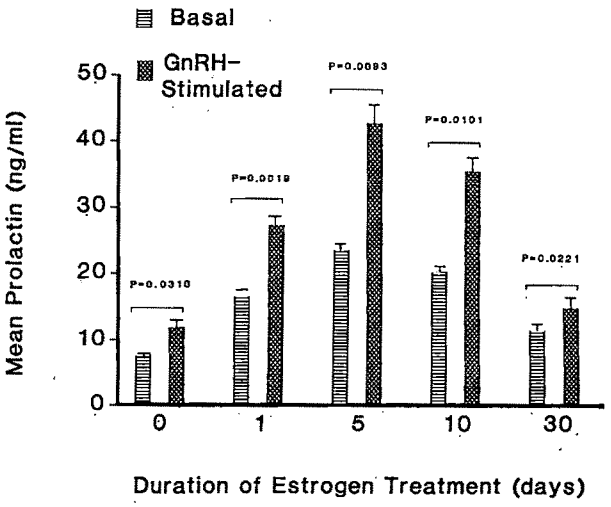


Fig. 4. Mean serum prolactin concentrations *(ng/ml)* in the estrogen-depleted state (day 0) and on days 1, 5, 10, and 30 of estrogen replacement in postmenopausal women. Blood samples were drawn every 15 minutes for 4 hours on each occasion. The first 2 hours represent basal conditions *(line bars)*, and the last 2 hours represent after-intravenous administration of 10 μg of GnRH *(fence bars)*.

the blood samples have previously been reported.[13] Serum estradiol was measured by specific radioimmunoassay as described.[16]

Data analysis. The effects of estrogen on prolactin concentrations in different sessions (days 0, 1, 5, 10, and 30) were assessed by analysis of variance with Duncan's multiple range test. Comparison of prolactin secretion basally and in response to GnRH stimulation was made by paired Student's *t* test. Correlations between serum estradiol and mean prolactin concentrations basally and in response to GnRH were sought by linear regression. Linear regression was also used to test for significant correlations between (1) mean increments in serum prolactin and the corresponding FSH, LH, and FSH/LH levels on each of the study days and (2) percent of maximal prolactin increments and percent of maximal FSH, LH, and FSH/LH increments.

Results

Serum estradiol. Before placement of the vaginal ring that contained estradiol, the mean serum estradiol concentration was 14.1 ± 3.5 pg/ml ± SEM. After the first 24 hours of estrogen treatment, this value increased significantly ($p < 0.0001$) to a maximum of 340 ± 44.2. Serum estradiol levels decreased gradually during the rest of the treatment period with concentrations of 253 ± 29.2 (day 5), 213 ± 26.2 (day 10), and 158 ± 24.5 (day 30), all of which were significantly elevated compared with levels before treatment ($p = 0.0001$).

Basal and GnRH-stimulated prolactin release and effect of estradiol. Fig. 1 shows the temporal profiles of mean serum prolactin levels derived from eight postmenopausal women who had blood samples taken at 15-minute intervals for 4 hours (beginning at 8:00) before estrogen treatment (day 0) and on days 1, 5, 10, and 30 of estradiol administration. A GnRH bolus injection was given at 10:00 on each study day as indicated.

Mean basal serum prolactin levels were obtained for the 2-hour intervals preceding administration of GnRH (8:00 to 10:00) for each of the five study sessions. As shown in the top panel of Fig. 2, basal serum prolactin concentrations increased significantly within 24 hours of estrogen treatment ($p = 0.005$). Although there was a trend toward an enhanced prolactin concentration on day 5, there were no significant differences between days 1, 5, and 10. By day 30 of estrogen treatment, the mean basal serum prolactin concentration was between the levels seen basally and on day 1. The circulating estradiol concentration was significantly and positively correlated ($r^2 = 0.266$; $p = 0.0011$) with mean basal serum prolactin levels during the entire time course (Fig. 3).

Mean levels of serum prolactin obtained after the GnRH bolus injection (10:15 to 12:00) on each of the 5 days of the study are shown in the bottom panel of Fig. 2. Compared with levels obtained before treatment with estrogen, mean serum GnRH-stimulated prolactin levels increased significantly ($p = 0.027$) after 24 hours of estrogen treatment, and this increase was followed by an additional increase by day 5 ($p = 0.026$). There

Table I. Mean increments above basal in prolactin, FSH, LH, and FSH/LH levels after GnRH stimulation

Estrogen treatment (days)	*Prolactin (ng/ml)*	*LH (mIU/ml)*	*FSH (mIU/ml)*	*FSH/LH*
0	4.6 ± 1.7[a]	41.3 ± 6.3[a]	23.6 ± 4.0[a]	0.54 ± 0.03[a]
1	10.7 ± 2.2[a,b]	33.2 ± 6.5[a]	17.4 ± 2.9[a]	0.54 ± 0.04[a]
5	19.3 ± 5.4[b]	37.8 ± 5.3[a]	20.6 ± 2.5[a]	0.56 ± 0.04[a]
10	17.1 ± 4.2[b]	42.0 ± 4.0[a]	20.6 ± 2.5[a]	0.48 ± 0.03[a]
30	8.4 ± 2.7[a,b]	21.6 ± 4.6[a]	14.8 ± 2.5[a]	0.74 ± 0.05[b]

All values are mean ± SEM. Differing superscripts (a,b) denote significant differences ($p < 0.05$) within columns by analysis of variance.

was no difference between GnRH-stimulated prolactin levels on days 5 and 10 or on days 1 and 10. By day 30 of estrogen treatment, the prolactin response to GnRH was similar to that obtained before administration of estrogen and that on day 1. A significant positive correlation was found between the ambient levels of serum estradiol and mean serum prolactin concentrations after GnRH stimulation ($r^2 = 0.181$; $p = 0.0078$; data not shown).

Comparison of basal and GnRH-stimulated prolactin, LH, and FSH release. Administration of GnRH induced a significant increase in mean serum prolactin concentrations on all study days (Fig. 4). However, the mean prolactin increments above basal were significantly higher ($p = 0.031$) on days 5 and 10 of estrogen treatment compared with those obtained in the estrogen-depleted state (Table I). Also shown in Table I are mean increments above basal in FSH, LH, and FSH/LH levels after administration of GnRH during each of the five study sessions. There was no significant difference in mean increments of FSH and LH among study days, but the FSH/LH level was higher on day 30 of estrogen treatment than on any other study day. To compare the prolactin and gonadotropin release after administration of GnRH, linear regression analysis was done between mean prolactin increments and the corresponding mean FSH, LH, and FSH/LH increments on individual study days. No significant correlations were obtained in either the estrogen-depleted state or during estrogen treatment. Likewise, we were unable to document any correlations between maximal percent of increments in serum prolactin concentrations and the corresponding FSH, LH, and FSH/LH levels (data not shown).

Comment

The present study confirms previous observations in postmenopausal women that administration of estrogen is associated with an increase in basal serum prolactin concentrations[5, 7] and that GnRH stimulation can effect an increase in circulating prolactin in the estrogen-deprived woman.[8, 10] In addition, we present evidence that administration of estrogen to postmenopausal women potentiates the prolactin response to GnRH stimulation without resulting in a parallel increase in gonadotropin release.

After reaching maximal concentrations within 24 hours after placement of the vaginal ring, serum estradiol levels decreased by day 5 of treatment, and throughout the rest of the treatment period they remained in the range typically observed during the late follicular phase of the menstrual cycle.[12, 13] The rise in circulating prolactin was prompt, increasing twofold after 24 hours of treatment and reaching a peak by day 5. Although we found a highly significant positive correlation between circulating concentrations of estradiol and prolactin, there appeared to be a time lag in the peak concentrations of the two hormones. Our results provide a detailed time course, which is in general agreement with the data obtained by Yen et al.[5] in four hypogonadal women, and showed an almost fourfold increase in serum prolactin levels after 2 weeks of orally administered ethinyl estradiol (1μg/kg/day). Moreover, our results on day 10 of estradiol administration are consistent with those of Joseph et al.,[7] who recently described a threefold increment in circulating prolactin levels after 10 days of intramuscular administration of estradiol benzoate (0.625 mg/day) in 10 postmenopausal women.

GnRH clearly stimulated prolactin secretion in the estrogen-depleted state. During estrogen treatment an enhanced prolactin response to GnRH was observed, which suggests that estrogen potentiates the prolactin-stimulating effect of GnRH. When Yen et al.[8] compared the effect of a GnRH agonist on circulating prolactin levels in hypogonadal women and in women at random stages of the menstrual cycle, no difference in the magnitude of prolactin response to the agonist was found between the two groups. In a later study Zhang and Yen[9] demonstrated that whereas a GnRH agonist elicited a significant increase in serum prolactin concentrations in postmenopausal women, no prolactin-stimulating effect of the agonist was found in normal young men. More recently, Mais and Yen[10] described a significant prolactin increment after administration of GnRH (10 μg intravenously) in a group of hypergo-

nadotropic hypogonadal women. In contrast, two of seven women in the early follicular phase of the menstrual cycle had a small response, even though the circulating estradiol levels were similar in the two groups. Accordingly, it is possible that the differences in prolactin responsiveness to the administration of GnRH may be attributed to intrinsic modulating factors such as endogenous GnRH secretion or the prevailing levels of sex steroid hormones.[17] We found not only that administration of estrogen potentiates the prolactin response to GnRH stimulation but also that there is a significant positive correlation between the serum estradiol concentration and GnRH-stimulated prolactin release. A likely role for sex steroids as modulators of the prolactin response to GnRH is also supported by the findings of Barbarino et al.,[4] who administered estrogen intramuscularly over a 9-day period to castrated and noncastrated men and measured the prolactin reponse to GnRH stimulation before and on the last day of treatment. No change in prolactin concentrations was noted in the absence of estrogen, whereas estrogen treatment induced a significant increase in the serum prolactin concentration, which was greater in the castrated than in the noncastrated men.

Although the mechanisms subserving the estrogen-associated elevation in circulating levels of prolactin remain ill-defined, they may involve a direct effect on prolactin synthesis[18] and/or lactotrope hyperplasia. Similarly, the process through which GnRH stimulates prolactin release is subject to conjecture. It has been suggested that GnRH may affect prolactin release through a central catecholaminergic mechanism[19] or via direct stimulation of pituitary lactotropes. However, one of the more intriguing mechanisms proposed is that of a paracrine interaction between the gonadotropes and the lactotropes.[11] In vitro studies have demonstrated that gonadotropes secrete a substance or substances with prolactin-releasing activity, the nature of which has yet to be determined.[11] A role for LH α-subunit as a possible mediator in GnRH induction of lactotropes has been suggested by Begeot et al.[20] However, more recent studies by Mais and Yen[10] have focused on a population of FSH-enriched gonadotropes as putative generators of a paracrine action on lactotropes. They found a significant positive correlation between maximal percent of prolactin increments after administration of GnRH (10 μg intravenously) and the ratio of FSH to LH maximal percent of increments in six hypergonadotropic hypogonadal women. Our results are at variance with this observation in that we were unable to demonstrate any correlation between prolactin and FSH to LH ratio with respect to mean increments or maximal percent of increments after a similar GnRH bolus injection in eight hypoestrogenic and estrogen-treated postmenopausal women studied serially after estradiol replacement. In addition, we found no correlation between GnRH-stimulated mean prolactin increments and mean increments in FSH or LH, which suggests that neither FSH nor LH release per se mediates the effect on GnRH on lactotropes. Our findings are supported by in vitro culture studies by Denef,[11] in which FSH and LH did not directly influence prolactin release.

We thank Donald L. Kaiser for valuable help with the statistical analyses; David Boyd for assistance with CLINFO; Sandra Jackson and her nursing staff at the Clinical Research Center; E. Elizabeth Taylor, Rebecca Weaver, Catherine Cassandra, and Anthony Amos for technical support; and Kay Hancock for help in preparing the manuscript.

REFERENCES

1. Djahanbakhch O, McNeilly AS, Warner PM, Swanston IA, Baird DT. Changes in plasma levels of prolactin, in relation to those of FSH, oestradiol, androstendione and progesterone around the preovulatory surge of LH in women. Clin Endocrinol 1984;20:463-72.
2. Robyn C, Delvoye P, Nokin J, et al. In: Pasteels JL, Robyn C, eds. Human prolactin. Amsterdam: Excerpta Med Found, International Congressional Series No. 308, 1973:167-88.
3. Frantz AG, Kleinberg DL, Noel GL. Studies on prolactin in man. Recent Prog Horm Res 1972;28:527-90.
4. Barbarino A, De Marinis L, Mancini A, Farabegoli C. Estrogen-dependent plasma prolactin response to gonadotropin-releasing hormone in intact and castrated men. J Clin Endocrinol Metab 1982;55:1212-6.
5. Yen SSC, Ehara Y, Siler TM. Augmentation of prolactin secretion by estrogen in hypogonadal women. J Clin Invest 1974;53:652-5.
6. Ehara Y, Siler TM, Yen SSC. Effects of large doses of estrogen on prolactin and growth hormone release. AM J OBSTET GYNECOL 1976;125:455-8.
7. Jean Joseph P, Couzinet B, Brailly S, Rigaud C, Raynaud JP, Schaison G. Interactions of oestradiol benzoate and promegestone upon basal and TRH-induced prolactin secretion in postmenopausal women. Clin Endocrinol 1986;24:497-503.
8. Yen SSC, Hoff JD, Lasley BL, Casper RF, Sheehan K. Induction of prolactin release by LRF and LRF-agonist. Life Sci 1980;26:1963-7.
9. Zhang Y-W, Yen SSC. Prolactin-releasing action of LRF: a central catecholamine mediated event? Life Sci 1984; 34:653-7.
10. Mais V, Yen SSC. Prolactin-releasing action of gonadotropin-releasing hormone in hypogonadal women. J Clin Endocrinol Metab 1986;62:1089-92.
11. Denef C. Paracrine interactions in the anterior pituitary. Clin Endocrinol Metab 1986;15:1-32.
12. Veldhuis JD, Samojlik E, Evans WS, et al. Endocrine impact of pure estradiol replacement in postmenopausal women: alterations in anterior pituitary hormone release and circulating sex steroid hormone concentrations. AM J OBSTET GYNECOL 1986;155:334-9.
13. Veldhuis JD, Evans WS, Rogol AD, Kolp L, Thorner MO, Stumpf P. The pituitary self-priming actions of gonadotropin-releasing hormone: kinetics of estradiol's potentiating effects on GnRH-facilitated LH and FSH release in healthy post-menopausal women. J Clin Invest 1986;77:1849-56.
14. Stumpf PG, Maruca J, Santen RJ, Demers LM. Development of a vaginal ring for achieving physiologic levels

of 17 beta-estradiol in hypoestrogenic women. J Clin Endocrinol Metab 1982;54:208-10.
15. Thorner MO, Perryman RL, Cronin MJ, et al. Successful treatment of acromegaly by removal of a pancreatic islet tumor secreting a growth hormone-releasing factor. J Clin Invest 1982;70:965-77.
16. Samojlik E, Veldhuis JD, Wells SA, Santen RJ. Preservation of androgen secretion during estrogen suppression with aminoglutethimide in the treatment of metastatic breast carcinoma. J Clin Invest 1980;65:602-12.
17. Gooren L, van der Veen EA, Kessel H. Modulation of prolactin secretion by gonadal steroids in men. In: MacLeod RM, Scapagnini U, eds. Central and peripheral regulation of prolactin function. New York: Raven Press, 1980:373-5.
18. Maurer RA. Relationship between estradiol, ergocryptine, and thyroid hormone: effects on prolactin synthesis and prolactin messenger ribonucleic acid levels. Endocrinology 1982;110:1515-20.
19. Van Loon GR. Bromocriptine-induced inhibition of plasma dopamine, noradrenaline and adrenaline responses to LH-RF. Nature 1978;275:331-2.
20. Begeot M, Hemming FJ, Dubois PM, Combarnous Y, Dubois MP, Aubert ML. Induction of pituitary lactotrope differentiation by luteinizing hormone alpha subunit. Science 1984;226:566-8.

Increased thromboxane biosynthesis in normal pregnancy is mainly derived from platelets

D. J. Fitzgerald, M.B., G. Mayo, R.N., F. Catella, M.D., S. S. Entman, M.D., and G. A. FitzGerald, M.D.

Nashville, Tennessee

Thromboxane biosynthesis was determined in normal pregnant subjects by measurement of its major urinary and plasma metabolites, 2,3-dinor-thromboxane B_2 and 11-dehydro-thromboxane B_2. Urinary 2,3-dinor-thromboxane B_2 increased early in pregnancy (731 ± 124 pg/mg creatinine) compared with nonpregnancy (<350 pg/mg creatinine; $p < 0.001$) and the postpartum period (155 ± 42 pg/mg creatinine, $p = 0.015$) and remained elevated throughout gestation. Similarly, plasma and urinary 11-dehydro-thromboxane B_2 were increased in pregnancy. To determine the cellular origin of the increase in thromboxane biosynthesis in pregnancy, platelet cyclooxygenase was selectively inhibited with aspirin in a dose of 120 mg orally followed by 20 mg twice daily for 7 days ($n = 4$). Selectivity was confirmed by measurement of urinary 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$, an index of prostacyclin biosynthesis. Coincident with a 97% inhibition of serum thromboxane B_2, urinary 2,3-dinor-thromboxane B_2 was almost completely inhibited and paralleled the recovery of platelet cyclooxygenase after withdrawal of aspirin. This study demonstrates that thromboxane biosynthesis is increased in pregnancy. The increase is mainly platelet derived and is consistent with increased platelet activation throughout pregnancy. (Am J Obstet Gynecol 1987;157:325-30.)

Key words: Pregnancy, thromboxane, prostacyclin, platelets

Platelet activation is enhanced in normal pregnancy as part of a generalized coagulopathy resulting in an increased risk of thrombosis.[1] What mediates the increase in platelet activity in pregnancy is unknown. Formation of thromboxane A_2, the major cyclooxygenase product of arachidonic acid in platelets and a potent platelet aggregant,[2] is reported to be increased in pregnancy.[3, 4] A possible source of increased thromboxane formation in pregnancy is the placenta, which has been shown to generate thromboxane in vitro.[5] However, estimates of thromboxane formation in pregnancy have been based on measurement of plasma immunoreactive thromboxane B_2, the hydrolysis product of thromboxane A_2. This approach is highly subject to both sampling and analytical artifact.[6] If thromboxane biosynthesis is indeed increased during pregnancy, an alternative source might be the platelet, the predominant origin of thromboxane A_2 generation in nonpregnant individuals.[7] We have previously demonstrated increased thromboxane formation in a number of conditions associated with platelet activation, including severe ath-

From the Division of Clinical Pharmacology and the Department of Obstetrics, Vanderbilt University.
This work was supported by Grant HD 17413 from the National Institutes of Health. Dr. D. J. Fitzgerald is the recipient of a Faculty Development Award from the Pharmaceutical Manufacturers Association Foundation. Dr. F. Catella holds a fellowship from the Tennessee Affiliate of the American Heart Foundation. Dr. G. A. FitzGerald is an Established Investigator of the American Heart Association.
Received for publication November 3, 1986; accepted March 5, 1987.
Reprint requests: Dr. Desmond Fitzgerald, Division of Clinical Pharmacology, Vanderbilt University, Nashville, TN 37232.

erosclerosis and unstable angina.[8] Platelet activation is also frequently associated with an increase in the formation of prostacyclin,[8, 9] the major cyclooxygenase products of arachidonic acid in endothelium and a potent inhibitor of platelet aggregation.[10] This increase in prostacyclin formation may represent a local compensatory mechanism by vascular endothelium to platelet activation. We have previously reported an increase in prostacyclin formation in normal pregnancy.[11] Although prostacyclin can be generated by the placenta,[5] the source of the increment in prostacyclin biosynthesis during pregnancy is uncertain. It is possible that it represents a vascular response to platelet activation.

In the present study we prospectively determined thromboxane A_2 biosynthesis in normal pregnancy by measurement of its major urinary enzymatic metabolites, 2,3-dinor-thromboxane B_2 and 11-dehydro-thromboxane B_2,[12, 13] and by measurement of its most abundant plasma metabolite, 11-dehydro-thromboxane B_2. Measurement of these metabolites avoids the problems of invasive sampling.[6, 13] Furthermore, the physicochemical methods that we used are highly sensitive and specific and avoid the analytic errors inherent in assays based on immunologic measurements.[6] To address the cellular origin of thromboxane biosynthesis during pregnancy we used selective inhibition of platelet cyclooxygenase.

Methods

The study group consisted of pregnant subjects attending the obstetrics clinic at Vanderbilt University prior to 20 weeks' gestation. All subjects gave informed written consent and the study was approved by the Committee for the Protection of Human Subjects at Vanderbilt University. All subjects were healthy at the time of admission into the study. Patients with a previous history of diabetes or renal disease were excluded. All subjects were asked to refrain from taking any medications during the study, in particular, aspirin or other nonsteroidal anti-inflammatory drugs. Patients attended the clinic at 2- to 4-week intervals. At each visit urine was analyzed for protein (Dipstick) and blood pressure was recorded in the sitting position using a standard mercury sphygmomanometer, with phase V of the Korotkoff sounds being recorded as the diastolic end point. Patients were excluded if they developed hypertension, diabetes, or intrauterine growth retardation. Collections of 24-hour urine samples were made at <20 weeks, at 20 to 28 weeks, at 28 weeks to term, and after 6 weeks post partum for determination of the urinary thromboxane metabolite 2,3-dinor-thromboxane B_2. Samples from the last trimester and the postpartum period were also analyzed for urinary 11-dehydro-thromboxane B_2. In a subset of patients blood was withdrawn in the third trimester with an 18-gauge needle in a syringe containing heparin, 100 units/ml, for determination of plasma 11-dehydro-thromboxane B_2, the most abundant metabolite of thromboxane B_2 in plasma.

To determine the major source of thromboxane A_2 biosynthesis in pregnancy, aspirin was administered orally in a loading dose of 120 mg followed by 20 mg twice daily for 7 days at 32 to 36 weeks' gestation in four normotensive pregnant women. Excretion of 2,3-dinor-thromboxane B_2 and 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ was determined in 24-hour urine samples before and during drug administration and during the 7-day period after withdrawal of aspirin. Similarly, serum thromboxane B_2 was determined in peripheral venous samples before and during aspirin administration and after drug withdrawal.

Biochemical analysis. Urine was analyzed for 2,3-dinor-thromboxane B_2 and 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ by combined gas chromatography–negative ion chemical ionization–mass spectrometry with their tetradeuterated isotopes used as internal standards. For determination of 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$, 5 ng of tetradeuterated standard was added to a 5 ml aliquot of urine and the sample was stored at −20° C until it was analyzed. After extraction and back extraction under alkaline and acidic conditions, the sample was derivatized as the methoxime, pentafluorobenzyl ester. After further purification on silica gel thin-layer chromatography platelets (Whatman Chemical Separation Inc., Clifton, New Jersey) derivatization was completed by formation of the trimethylsilyl ether derivative. Quantitation was accomplished by stable isotope dilution with a Hewlett Packard 5980 instrument operated in the negative ion mode, monitoring m/z 586 for endogenous 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ and m/z 590 for the deuterium-labeled internal standard. For determination of 2,3-dinor-thromboxane B_2, 7.25 ng of internal standard was added to a 5 ml aliquot of urine and the sample was derivatized as the methoxime derivative. After extraction by bonded-phase phenyl boronic acid column and further purification by C-18 Sep-Pak (Waters Associates, Milford, Massachusetts) and thin-layer chromatography the sample was derivatized as the methoxime, pentafluorobenzyl ester. Derivatization was completed after further thin-layer chromatography by formation of the trimethysilyl ether. Analysis was by gas chromatography–negative ion chemical ionization–mass spectrometry with the use of a capillary column interfaced with a Nermag R10-10C and monitoring m/z 586.5 for endogenous 2,3-dinor-thromboxane B_2 and m/z 590.5 for the tetradeuterated internal standard. Repeat measurements of 2,3-dinor-

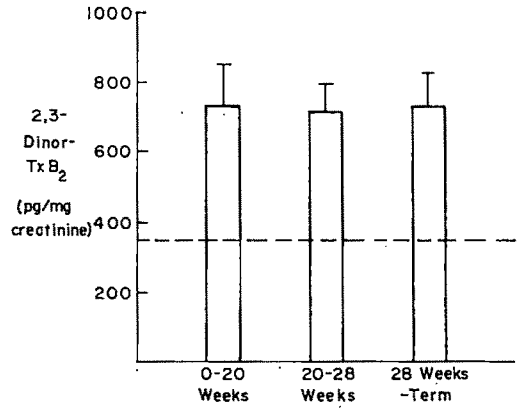


Fig. 1. Urinary excretion of 2,3-dinor-thromboxane B_2 *(TxB₂)* in pregnant subjects (n = 18) throughout gestation compared with nonpregnant subjects ($p < 0.001$). The *hatched line* represents the upper limit of normal (95% upper confidence limit) in normal nonpregnant women.

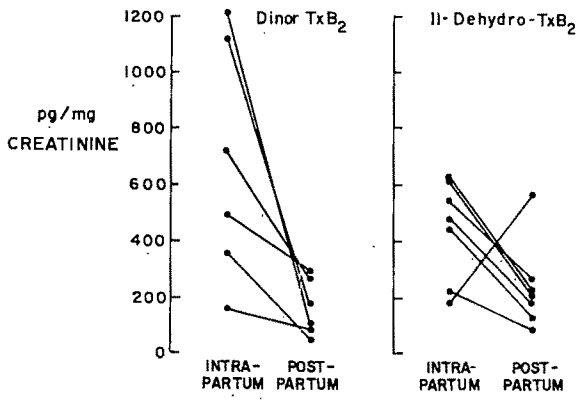


Fig. 2. Comparison of urinary 2,3-dinor-thromboxane B_2 *(Dinor TxB₂)* (n = 6; p = 0.015) and 11-dehydro-thromboxane B_2 *(11-Dehydro-TxB₂)* (n = 7; p = 0.029) in the last trimester and in the postpartum period.

thromboxane B_2 in 24-hour urine samples collected over five successive days in four pregnant subjects showed good day-to-day reproducibility with a coefficient of variation of 23% ± 9%.

Plasma was analyzed for 11-dehydro-thromboxane B_2 according to methods similar to those used for determining urinary metabolites.[13] Briefly, 5 ng of deuterated internal standard was added to 10 ml of plasma. After acidification, the sample was extracted by C-18 Sep-Pak, eluted with ethyl acetate and further purified by thin-layer chromatography. The sample was derivatized as the pentafluorobenzyl ester and after a further thin-layer chromatography step derivatization was completed by trimethylsilylation. Quantitation was as for 2,3-dinor-thromboxane B_2 monitoring m/z 511 for endogenous 11-dehydro-thromboxane B_2 and m/z 515 for the tetradeuterated internal standard.

Serum thromboxane B_2 was determined by a modification of the method of Fitzpatrick et al.[14] after incubation of 3 ml of whole blood in a glass test tube at 37° C for 45 minutes.

Statistical analysis. All data are expressed as the mean ± SEM. The data were analyzed by Kruskal-Wallis analysis of variance and subsequent comparison between groups was performed by the Wilcoxon rank-sum test for paired and unpaired data as appropriate. These are nonparametric tests that avoid assumptions as to the distribution of the data.

Results

Eighteen patients, seven black and 11 white, with a mean age of 22.3 ± 1.0 years were prospectively studied throughout gestation. Nine of our subjects were primigravid. All of our subjects remained normotensive and had normal deliveries with a mean birth weight of 7.17 ± 0.3 pounds. Urinary 2,3-dinor-thromboxane B_2 was increased at less than 20 weeks (731 ± 124 pg/mg of creatinine) and remained elevated throughout pregnancy (714 ± 81 and 726 ± 104 pg/mg of creatinine, respectively, at 20 to 28 weeks and 28 weeks to term) when compared with normal nonpregnant levels (<350 pg/mg of creatinine; $p < 0.001$, Fig. 1) or with levels in the postpartum period (155 ± 42 pg/mg of creatinine; $p = 0.015$, Fig. 2). In addition to an increase in 2,3-dinor-thromboxane B_2, urinary excretion of 11-dehydro-thromboxane B_2 was increased in all but one pregnant subject studied in the third trimester (440 ± 67 pg/mg creatinine) when compared with the level found in urine collected at more than 6 weeks post partum (233 ± 59 pg/mg of creatinine; $p = 0.029$, Fig. 2). Consistent with this observation, plasma 11-dehydro-thromboxane B_2 was increased in the third trimester when compared with levels found in nonpregnant normal subjects (3.2 ± 0.76 versus 1.3 ± 0.16 pg/ml; $p = 0.016$, Fig. 3).

Administration of aspirin was associated with a marked reduction in serum thromboxane B_2, the peak effect occurring on day 7 with a mean inhibition of 97% from pretreatment levels (Fig. 4). Withdrawal of aspirin resulted in the gradual recovery of platelet cyclooxygenase with a mean inhibition of only 13% of serum thromboxane B_2 by 7 days after withdrawal. Consistent with the platelet being the major source of thromboxane biosynthesis in vivo in these subjects, urinary 2,3-dinor-thromboxane B_2 followed a similar pattern, with a mean inhibition of 94% by day 7 of aspirin therapy and a gradual recovery after aspirin withdrawal (Fig. 4). Excretion of 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ was increased (mean and range, 1564 and 531 to 3505 pg/mg of creatinine) in all four subjects

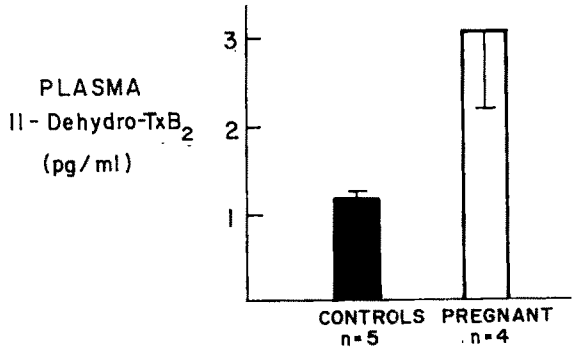


Fig. 3. Comparison of plasma 11-dehydro-thromboxane B_2 *(11-Dehydro-TxB$_2$)* in pregnant (n = 4) and nonpregnant (n = 5; p = 0.016) subjects.

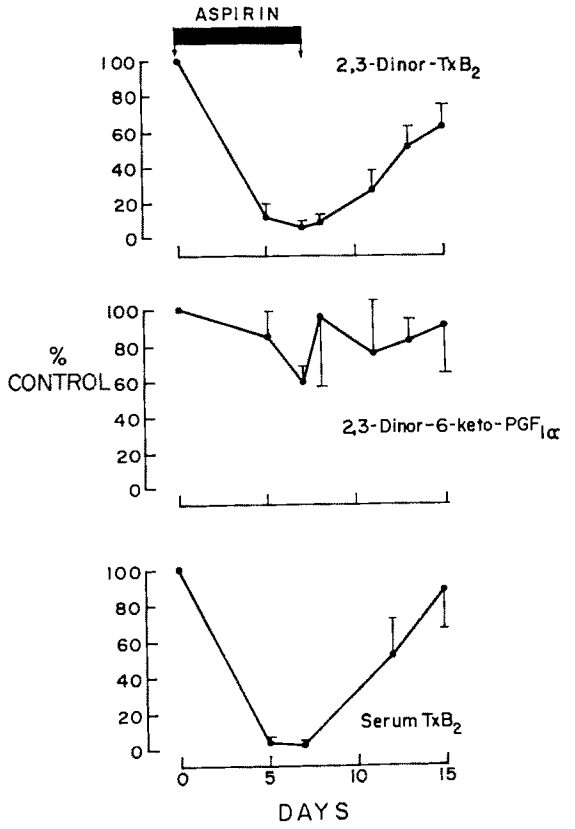


Fig. 4. Excretion of urinary 2,3-dinor-thromboxane B_2 *(2,3-Dinor-TxB$_2$)*, 2,3-dinor-prostaglandin $F_{1\alpha}$ *(2,3-Dinor-6-keto-PGF$_{1\alpha}$)* and serum thromboxane B_2 *(Serum TxB$_2$)* in normal pregnant subjects (n = 4) before, during, and after administration of 120 mg of aspirin orally followed by 20 mg twice daily for 7 days.

when compared with nonpregnant levels (<220 pg/mg creatinine), as is seen in normal pregnancy. Administration of aspirin resulted in a mean inhibition of 15% of urinary 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ by day 5 and a mean inhibition of 40% by day 7 of therapy. These changes did not, however, achieve statistical significance.

Comment

These studies demonstrate an increase in urinary 2,3-dinor-thromboxane B_2 in normal pregnant subjects that occurs early in and persists throughout gestation. Previous studies demonstrate that 2,3-dinor-thromboxane B_2 is a major urinary metabolite of thromboxane B_2, the nonenzymatic hydrolysis product of thromboxane A_2.[15] Thus these findings suggest that thromboxane biosynthesis is increased in normal pregnancy. To address the possibility that the increased excretion of 2,3-dinor-thromboxane B_2 reflected preferential metabolism of thromboxane B_2 to this metabolite in pregnancy and not an increase in thromboxane formation, we measured excretion of 11-dehydro-thromboxane B_2, which represents a second major pathway of thromboxane B_2 enzymatic metabolism.[12, 13] Consistent with the urinary 2,3-dinor-thromboxane B_2 data, excretion of 11-dehydro-thromboxane B_2 was increased in pregnant subjects compared with the levels found post partum. Another possible mechanism for the increase in urinary thromboxane metabolites is an increase in renal clearance during pregnancy. For this reason the data were corrected for urinary creatinine. In addition, plasma 11-dehydro-thromboxane B_2 was also increased in pregnancy, which demonstrates that the increase in urinary metabolites reflected increased formation and not simply an increase in their renal clearance. In any event, at steady state, urinary excretion of thromboxane B_2 metabolites will equal their generation and will be independent of renal function. Thus the increase in 2,3-dinor-thromboxane B_2 excretion in our subjects reflected an increase in thromboxane biosynthesis in pregnancy and not an alteration in thromboxane B_2 metabolism or renal function.

In nonpregnant subjects, the major source of urinary 2,3-dinor-thromboxane B_2 is platelet-derived thromboxane.[7] Thus selective inhibition of platelet cyclooxygenase results in a marked reduction in urinary 2,3-dinor-thromboxane B_2 excretion.[7] Similar studies suggest that 11-dehydro-thromboxane B_2 in urine is also mainly derived from platelet thromboxane (Catella F, FitzGerald GA, unpublished data). It has been suggested that the placenta forms thromboxane and that this may play a role in modulating umbilical blood flow.[3] Thus the increase in thromboxane formation in pregnancy may be partly derived from the placenta. To determine the predominant source of the increment in thromboxane formation in pregnancy we examined thromboxane metabolite excretion after administration of a low-dose aspirin regimen that would selectively inhibit platelet cyclooxygenase. Inhibition of platelet

cyclooxygenase was monitored by determination of serum thromboxane B_2, a measure of the capacity of platelets to generate thromboxane A_2. After 5 days of treatment, serum thromboxane B_2 was almost completely inhibited. Coincident with the inhibition of platelet cyclooxygenase, there was a marked reduction in 2,3-dinor-thromboxane B_2 excretion. That this reflected inhibition of platelet cyclooxygenase was supported by two additional findings. First, excretion of 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$, the major urinary metabolite of prostacyclin,[6] was inhibited minimally and only transiently. Urinary 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ is increased in normal pregnancy, which reflects an increase in prostacyclin biosynthesis[11] and is an index of tissue cyclooxygenase activity. Thus this dose of aspirin appears to be relatively selective for platelet cyclooxygenase. Second, the recovery of 2,3-dinor-thromboxane B_2 excretion paralleled the recovery of platelet cyclooxygenase. Aspirin irreversibly inhibits the cyclooxygenase enzyme so that recovery is dependent on the formation of new enzyme.[16] Tissue cyclooxygenase recovers rapidly through formation of new protein. However, the anucleate platelet cannot generate new enzyme so that recovery of platelet cyclooxygenase and therefore of serum thromboxane B_2 is dependent on the generation of new platelets. Since platelets have a half-life of about 5 days, serum thromboxane B_2 increases gradually after aspirin withdrawal, returning to normal at about 10 days.[17] Thus the delayed recovery of urinary 2,3-dinor-thromboxane B_2 after aspirin withdrawal in our subjects is consistent with the platelet being the major source of the increase in thromboxane biosynthesis in pregnancy.

We have demonstrated previously that thromboxane biosynthesis is increased in conditions associated with platelet activation.[8] Although some studies have suggested that platelet function is normal in uncomplicated pregnancy,[18] others have reported changes consistent with in vivo platelet activation.[1] Thus circulating platelet aggregates are increased[19] and plasma levels of the platelet granule constituent β-thromboglobulin are elevated.[20] In addition, recovery of platelet cyclooxygenase activity after a single dose of aspirin is slightly accelerated[21] and platelet volume is increased,[22] both of which are consistent with increased platelet turnover. Studies of ex vivo platelet function in pregnancy demonstrate hypoaggregability suggesting that platelets have been partially activated in vivo.[1] Thus the increase in thromboxane biosynthesis in pregnancy probably reflects increased platelet activation. As in other conditions in which there is platelet activation,[8] prostacyclin formation is increased in pregnancy. However, it is unlikely that this is due mainly to platelet activation since the increase in prostacyclin formation far exceeds the increase in thromboxane. Certainly, thromboxane-dependent platelet activation is unlikely to represent a major stimulus as prostacyclin biosynthesis is unaltered by selective inhibition of platelet cyclooxygenase by low-dose aspirin.

Previous studies have reported thromboxane formation to be increased, unchanged, or decreased in pregnancy.[3, 4] The difference between studies may in part reflect the analytic approaches that were used. A major difference between this and previous studies is that thromboxane biosynthesis was determined noninvasively with the use of methods that avoid sampling and analytic errors.[6] In many studies thromboxane formation is determined by measurement of thromboxane B_2 in plasma.[3, 4] However, the capacity of platelets to generate thromboxane exceeds by orders of magnitude the amount present in plasma. Therefore even minor degrees of platelet activation during sampling will induce formation of thromboxane A_2 and its nonenzymatic hydrolysis to thromboxane B_2. This will result in artifactually high levels of thromboxane B_2, which will obscure smaller physiologic changes.[13] Measurement of plasma and urinary enzymatic metabolites minimizes this sampling artifact.[6, 13] A second major source of error is the use of immunologic assays to measure prostanoids in the low picogram per milliliter range in biologic fluids. Cross-reactivity of the antibodies can give spurious results when one is attempting to measure minute concentrations in such systems, frequently overestimating what is present and obscuring changes in the prostanoid of interest.[6] In this study, urinary and plasma thromboxane metabolites were determined by direct physicochemical methods that avoid these analytic errors.

The role of the increase in thromboxane biosynthesis in modulating platelet activity in pregnancy is unknown. However, accelerated platelet activation precedes the development of pregnancy-induced hypertension.[23] In addition, Wallenburg et al.[24] and Beaufils et al.[25] have demonstrated that chronic inhibition of thromboxane formation with aspirin prevents the development of the disease and limits its sequelae. Together, these findings suggest a pathophysiologic role for thromboxane and platelet activation in the development of pregnancy-induced hypertension. In addition we have previously demonstrated that prostacyclin biosynthesis fails to increase normally in pregnant subjects who subsequently develop pregnancy-induced hypertension.[11] Thus the normal increase in prostacyclin formation that occurs in pregnancy may play a role in limiting platelet activation. It may be important if aspirin is to be of value in the prevention of pregnancy-induced hypertension to use a dose that inhibits platelet thromboxane formation but preserves the ability to generate prostacyclin. Although the dose of aspirin used by Wallenburg et al.[24] (60 mg) should be relatively

platelet selective, the effectiveness of this dose in inhibiting thromboxane formation and preserving prostacyclin biosynthesis was not assessed. Our studies suggest that an even smaller dose of aspirin will result in virtually complete inhibition of platelet cyclooxygenase while prostacyclin biosynthesis is maintained.

In conclusion, thromboxane biosynthesis is increased in pregnancy. The increase in thromboxane A_2 formation is mainly derived from platelets and is consistent with increased platelet activation throughout normal pregnancy.

REFERENCES

1. McKay D. Chronic intravascular coagulation in normal pregnancy and preeclampsia. Contrib Nephrol 1981;25:108.
2. Granström E, Diczfalusy U, Hamberg M, Malmsten C, Samuelsson B. Thromboxane A_2: biosynthesis and effect on platelets. Adv Prostaglandin Thromboxane Leukotriene Res 1982;10:15.
3. Ylikorkala O, Viinikka L. Thromboxane A_2 in pregnancy and the puerperium. Br Med J 1980;281:1601.
4. Mitchell MD, Bibby JG, Hicks BR, Redman CWG, Anderson ABM, Turnbull AC. Thromboxane A_2 and human parturition: concentrations in plasma and production *in vitro*. J Endocrinol 1978;78:435.
5. Walsh SW. Pre-eclampsia: an imbalance in placental prostacyclin and thromboxane production. AM J OBSTET GYNECOL 1985;152:335.
6. FitzGerald GA, Pedersen AK, Patrono C. Analysis of prostacyclin and thromboxane A_2 biosynthesis in cardiovascular disease. Circulation 1983;67:1174.
7. Reilly IAG, FitzGerald GA. Inhibition of thromboxane formation *in vivo* and *ex vivo*: implications for therapy with platelet inhibitory drugs. Blood 1987;69:180.
8. Fitzgerald DJ, Roy L, Catella F, FitzGerald GA. Platelet activation in unstable coronary disease. N Engl J Med 1986;315:983.
9. FitzGerald GA, Smith B, Pedersen AK, Brash AR. Prostacyclin biosynthesis is increased in patients with severe atherosclerosis and platelet activation. N Engl J Med 1984;310:1065.
10. Bunting SR, Gryglewski R, Moncada AS, Vane JR. Arterial walls generate from prostaglandin endoperoxides a substance (prostaglandin X) which relaxes strips of mesenteric and coeliac arteries and inhibits platelet aggregation. Prostaglandins 1976;12:897.
11. Fitzgerald DJ, Entman SS, Mulloy K, FitzGerald GA. Decreased prostacyclin biosynthesis preceding the clinical manifestations of pregnancy-induced hypertension. Circulation 1987;75:956.
12. Roberts LJ, Sweetman BJ, Oates JA. Metabolism of thromboxane B_2 in man: identification of twenty urinary metabolites. J Biol Chem 1981;256:8384.
13. Catella F, Healy D, Lawson J, FitzGerald GA. 11-Dehydro-TxB_2: a quantitative index of thromboxane A_2 biosynthesis in the human circulation. Proc Natl Acad Sci USA 1986;83:5861.
14. Fitzpatrick FA, Gorman RR, McGuire JC, Kelly RC, Wylanda MA, Sun FF. A radioimmunoassay for thromboxane B_2. Anal Biochem 1977;82:1.
15. Patrono C, Ciabattoni G, Pugliese F, Pierucci A, Blair IA, FitzGerald GA. Estimated rate of thromboxane secretion into the circulation of normal man. J Clin Invest 1986;77:590.
16. Roth GJ, Stanford A, Majerus PW. Acetylation of prostaglandin synthase by aspirin. Proc Natl Acad Sci USA 1975;72:3073.
17. Stuart M, Murphy S, Oski EA. A simple, non-radioisotopic technique for the determination of platelet lifespan. N Engl J Med 1975;292:1310.
18. Wallenburg HCS, van Kessel PH. Platelet lifespan in normal pregnancy as determined by a nonradioisotopic technique. Br J Obstet Gynaecol 1978;85:33.
19. O'Brien WF, Saba HI, Knuppel RA, Scerbo JC, Cohen GR. Alterations in platelet concentration and aggregation in normal pregnancy and preeclampsia. AM J OBSTET GYNECOL 1986;155:486.
20. Inglis TCM, Stuart J, George AJ, Davies AJ. Haemostatic and rheological changes in normal pregnancy and pre-eclampsia. Br J Haematol 1982;50:461.
21. Rakoczi CWG, Tallian Bagdany S, Gati I. Platelet lifespan in normal pregnancy and preelampsia as determined by a non-radioisotope technique. Thromb Res 1979;15:553.
22. Sill PR, Lind T, Walker W. Platelet values during normal pregnancy. Br J Obstet Gynaecol 1985;92:480.
23. Redman CWG, Bonnar J, Beilin L. Early platelet consumption in pre-eclampsia. Br Med J 1978;1:467.
24. Wallenburg HCS, Dekker GA, Makovitz JW, Rotmans P. Low-dose aspirin prevents pregnancy-induced hypertension and preeclampsia in angiotensin-sensitive primigravidae. Lancet 1986;1:1.
25. Beaufils M, Donsimoni R, Uzan S, Colau JC. Prevention of preeclampsia by early antiplatelet therapy. Lancet 1985;2:240.

Preeclampsia, delivery, and the hemostatic system

Abdelaziz A. Saleh, M.D., Ph.D., Sidney F. Bottoms, M.D., Robert A. Welch, M.D., Abdelkarim M. Ali, M.D., Federico G. Mariona, M.D., and Eberhard F. Mammen, M.D.
Detroit, Michigan

To determine the effects of preeclampsia and delivery, the hemostatic system was evaluated before and 24 to 48 hours after delivery in 59 nulliparous patients without clinical signs of disseminated intravascular coagulation. Fifteen patients with mild preeclampsia and 18 with severe preeclampsia were compared with 26 pregnant control patients. Preeclampsia was associated with high fibronectin ($p < 0.001$), low antithrombin III ($p < 0.001$), and low α_2-antiplasmin ($p < 0.005$), suggesting endothelial injury, clotting, and fibrinolysis, respectively. After delivery, fibronectin decreased only in preeclamptic patients ($p < 0.005$); α_2-antiplasmin increased in all groups ($p < 0.001$). Endothelial injury in preeclampsia appeared to resolve soon after delivery, which could contribute to the rapid clinical improvement noted in the early puerperium. (Am J Obstet Gynecol 1987;157:331-6.)

Key words: Preeclampsia, delivery, hemostatic system, fibronectin, antithrombin, antiplasmin

Recent advances in coagulation research have facilitated a better general understanding of the hemostatic system. The use of new techniques in the study of preeclampsia is of particular interest, since the cause of the occasionally associated disseminated intravascular coagulation (DIC) is unclear. However, pregnancy and delivery normally alter hemostasis, potentially confounding studies of preeclampsia.

There have been some studies with new markers for endothelial injury, clotting, and fibrinolysis to characterize hemostasis in pregnancy and/or preeclampsia. Fibronectin, a suggested marker for endothelial damage, is reported to be increased in preeclampsia.[1] Antithrombin III appears to be reduced in preeclampsia, which suggests enhanced clotting.[2,3] α_2-Antiplasmin determined by immunoassay is reported to be similar in normal pregnancy and preeclampsia, which suggests no increase in fibrinolysis.[4]

However, many questions regarding these new assays and hemostasis in pregnancy and/or preeclampsia remain unanswered. It is unclear from previous studies whether fibrin monomer, a marker for clotting, is unchanged or increased.[5,6] Assays of α_2-antiplasmin activity, more representative of biologic function than immunoassays, have not been used in pregnancy or preeclampsia. Likewise, D-dimer, which indicates the lysis of fibrin clots, has not been reported. With the exception of antithrombin III, modern coagulation technology has not been applied to determine the effect of delivery on hemostasis in normal pregnancy. Although delivery appears to be therapeutic for DIC associated with preeclampsia, none of the newer markers has been used to better understand this beneficial effect. Attempts to grasp the overall picture of hemostasis in pregnancy and preeclampsia are further hampered by the lack of studies that comprehensively evaluate the elements of hemostasis, such as platelets, endothelial injury, clotting, and fibrinolysis. Studies with new markers have rarely provided older assays, such as platelet count, fibrinogen, and fibrin(ogen) split products, to facilitate integration of new information into previously established models.

The purpose of this study is to apply recent advances in coagulation technology to comprehensively evaluate the effects of preeclampsia and delivery on hemostasis, while providing older assays for comparison.

Patients and methods

A total of 59 nulliparous patients, 33 with preeclampsia and 26 control patients of comparable gestational age were studied. Patients were chosen to exclude those with chronic hypertension, renal disease, abruptio placentae, chorioamnionitis, diabetes mellitus, multiple gestation, and other major medical and/or obstetric risks, except preeclampsia and preterm labor. The study was limited to patients without clinical signs of DIC; all cases with a bleeding diathesis, a platelet count $<100{,}000/mm^3$, a fibrinogen <100 mg/dl, an abnormally prolonged prothrombin time, or an abnormally prolonged partial thromboplastin time were excluded. During recruitment, investigators were contacted only when patients agreed to participate; the number who refused is not available. All patients indicated informed

From the Departments of Obstetrics and Gynecology, Surgery, Pathology, and Physiology, Wayne State University/Hutzel Hospital.
Received for publication July 14, 1986; revised April 16, 1987; accepted April 27, 1987.
Reprint requests: Sidney F. Bottoms, M.D., Hutzel Hospital, Department of Obstetrics and Gynecology, 4707 St. Antoine, Detroit, MI 48201.

Table I. Methods used for coagulation assays

Assay	*Method*	*Reference No.*
Platelet count and volume	Automated platelet analyzer*	8
Fibronectin	Nephelometry,† Boehringer Mannheim (Houston, Tex.) biochemica kits	9
Fibrinogen	Turbidimetry,† Pacific Hemostasis Laboratories (Ventura, Calif.) kits	8
Antithrombin III	Chromogenic substrate (Kabi S-2238)†	8
Fibrin monomer	Protamine sulfate, Dade Data Fi kits	8
α_2-Antiplasmin	Chromogenic substrate (Kabi S-2251)†	8
Fibrin(ogen) split products	Latex agglutination, Dade Data Fi kits	8
D-Dimer	Latex agglutination, American Diagnostics (Greenwich, Conn.) kits	10

*Baker Series 810.

†Multistat III centrifugal analyzer.

Table IIA. Clinical characteristics reported as mean (SD) of patients in labor

Preeclampsia	*None N = 20*		*Mild N = 11*		*Severe N = 13*	
Maternal age (yr)	22	(8)	20	(3)	20	(5)
Systolic blood pressure (mm Hg)	118	(9)	152	(20)	171	(13)
Diastolic blood pressure (mm Hg)	73	(9)	100	(9)	110	(6)
Proteinuria*	0	(0)	0.8	(0.6)	1.1	(1.4)
Gestational age (wk)	39	(2)	39	(2)	38	(2)
Birth weight (gm)	3100	(410)	3120	(620)	3000	(510)

*Semiquantitative dipstick recorded as 0 to 4+.

Table IIB. Clinical characteristics reported as mean (SD) of patients not in labor

Preeclampsia	*None N = 6*		*Mild N = 4*		*Severe N = 5*	
Maternal age (yr)	22	(2)	25	(6)	21	(5)
Systolic blood pressure (mm Hg)	108	(8)	155	(13)	178	(21)
Diastolic blood pressure (mm Hg)	63	(3)	100	(8)	109	(10)
Proteinuria*	0	(0)	1	(0.8)	2.2	(1.5)
Gestational age (wk)	38	(3)	39	(1)	34	(5)
Birth weight (gm)	2670	(690)	2820	(350)	1830	(970)

*Semiquantitative dipstick recorded as 0 to 4+.

consent on a form approved by the Human and Animal Investigation Committee of Wayne State University. Study and control patients were predominantly black and were indigent. Of the 59 gravidas, 15 had mild preeclampsia, 18 had severe preeclampsia, 44 were in labor, and 15 were not in labor. The criteria used to diagnose preeclampsia were those adopted by The American College of Obstetricians and Gynecologists.[7]

With a syringe that had a 21-gauge or larger butterfly needle, blood was obtained by venipuncture before delivery and 24 to 48 hours after delivery and was transferred into blood collection tubes (Becton Dickinson Vacutainer Systems, Rutherford, New Jersey). The first 3 ml of blood were placed in an ethylenediaminetetraacetic acid (EDTA) tube and used to measure platelet count and mean platelet volume. The next 9 ml of blood were drawn into a second syringe and transferred in equal volumes into a tube containing 0.5 ml of 3.2% sodium citrate and a second tube containing EDTA and aprotinin. Fibronectin was assayed in plasma from the second tube; all other assays were done on citrated plasma. Both tubes were centrifuged at 3000 × g for 30 minutes. Plasma was stored in small aliquots at −70° C for future analysis. The methods used for coagulation assays are outlined in Table I, and have been described in detail in previous publications.[8-10] Proteinuria was determined qualitatively (0 to 4+) with dipsticks.

Multivariate analysis of the covariance was used to evaluate the effects of preeclampsia, delivery, and their interaction on the various laboratory parameters of hemostasis, as well as the possibility of confounding factors related to labor or gestational age. Patients not in labor were believed to represent a different group from those in labor and were analyzed accordingly. Gestational age was analyzed as a covariate. Race and socio-

Table III. Parameters of hemostasis* for patients in labor reported as mean (SD)

	Preeclampsia									
	None (N = 20)				*Mild (N = 11)*				*Severe (N = 13)*			
	B		*A*		*B*		*A*		*B*		*A*	
Platelet count	220	(52)	233	(48)	248	(112)	238	(76)	235	(72)	227	(79)
Platelet volume	8.5	(1.1)	8.6	(0.8)	8.9	(2.1)	8.7	(2.0)	8.6	(0.8)	8.1	(0.8)
Fibronectin	251	(109)	261	(76)	359	(140)	282	(65)	399	(139)	279	(47)
Fibrinogen	549	(121)	604	(127)	602	(137)	566	(148)	642	(145)	630	(165)
Antithrombin III	97	(10)	99	(12)	94	(13)	96	(11)	94	(11)	89	(12)
Fibrin monomer	80	(41)	65	(49)	100	(0.0)	73	(47)	83	(37)	60	(50)
α_2-Antiplasmin	89	(16)	105	(19)	82	(12)	94	(19)	80	(17)	102	(17)
Fibrin(ogen) split products	28	(31)	59	(90)	71	(76)	35	(38)	91	(100)	36	(48)
D-Dimer	0	(0)	10	(45)	55	(25)	27	(47)	38	(65)	46	(77)

B = Before delivery; A = after delivery.

*Units are as follows: platelet count, thousands per cubic millimeter; platelet volume, cubic microns; fibronectin and fibrin(ogen) split products; antithrombin III and α_2-antiplasmin, percent of normal; fibrin monomer and D-dimer, percent of patients with detectable levels.

Table IV. Parameters of hemostasis for patients not in labor reported as mean (SD)

	Preeclampsia									
	None (N = 6)				*Mild (N = 4)*				*Severe (N = 5)*			
	B		*A*		*B*		*A*		*B*		*A*	
Platelet count	215	(36)	229	(42)	204	(53)	219	(38)	163	(69)	133	(66)
Platelet volume	8.3	(0.5)	8.1	(1.1)	9.2	(1.0)	9.0	(1.0)	9.4	(0.7)	9.1	(1.0)
Fibronectin	254	(54)	224	(47)	443	(87)	274	(51)	527	(104)	316	(83)
Fibrinogen	617	(160)	672	(163)	603	(100)	638	(243)	524	(240)	613	(191)
Antithrombin III	100	(10)	100	(13)	79	(13)	87	(10)	79	(5)	76	(4)
Fibrin monomer	67	(52)	100	(0)	100	(0)	50	(58)	100	(0)	60	(55)
α_2-Antiplasmin	97	(7)	117	(11)	71	(8)	101	(14)	84	(12)	95	(12)
Fibrin(ogen) split products	88	(94)	80	(55)	75	(40)	40	(30)	73	(44)	134	(112)
D-Dimer	0	(0)	33	(52)	25	(50)	25	(50)	40	(55)	80	(84)

See Table III for explanations.

Table V. Multivariate analysis of covariance of changes in hemostasis associated with preeclampsia, delivery, and their interaction (i.e., effects of delivery specific for preeclampsia)

	Preeclampsia	*Delivery*	*Interaction*
Overall	$p < 0.001$	$p < 0.001$	$p < 0.05$
Platelet count	NS	NS	NS
Platelet volume	NS	($p < 0.05$)	NS
Fibronectin	$p < 0.001$	$p < 0.001$	$p < 0.005$
Fibrinogen	NS	NS	NS
Antithrombin III	$p < 0.001$	NS	($p < 0.05$)
Fibrin monomer	NS	($p < 0.05$)	NS
α_2-Antiplasmin	$p < 0.005$	$p < 0.001$	NS
Fibrin(ogen) split products	NS	NS	NS
D-Dimer	NS	NS	NS

NS = Not significant.

economic status were not considered as confounding variables. The incidence of preeclampsia varies with these factors; controlling for them could result in underestimating the effect of preeclampsia. Equal weight was given to each cell in the analysis to avoid biases in detecting effects related only to sample size. Effects on the overall pattern of all nine parameters of hemostasis were considered significant for $p < 0.05$. In view of the multiple comparisons made in this study, effects on individual coagulation tests were considered significant for $p < 0.01$, and comparisons between individual cells were not made.

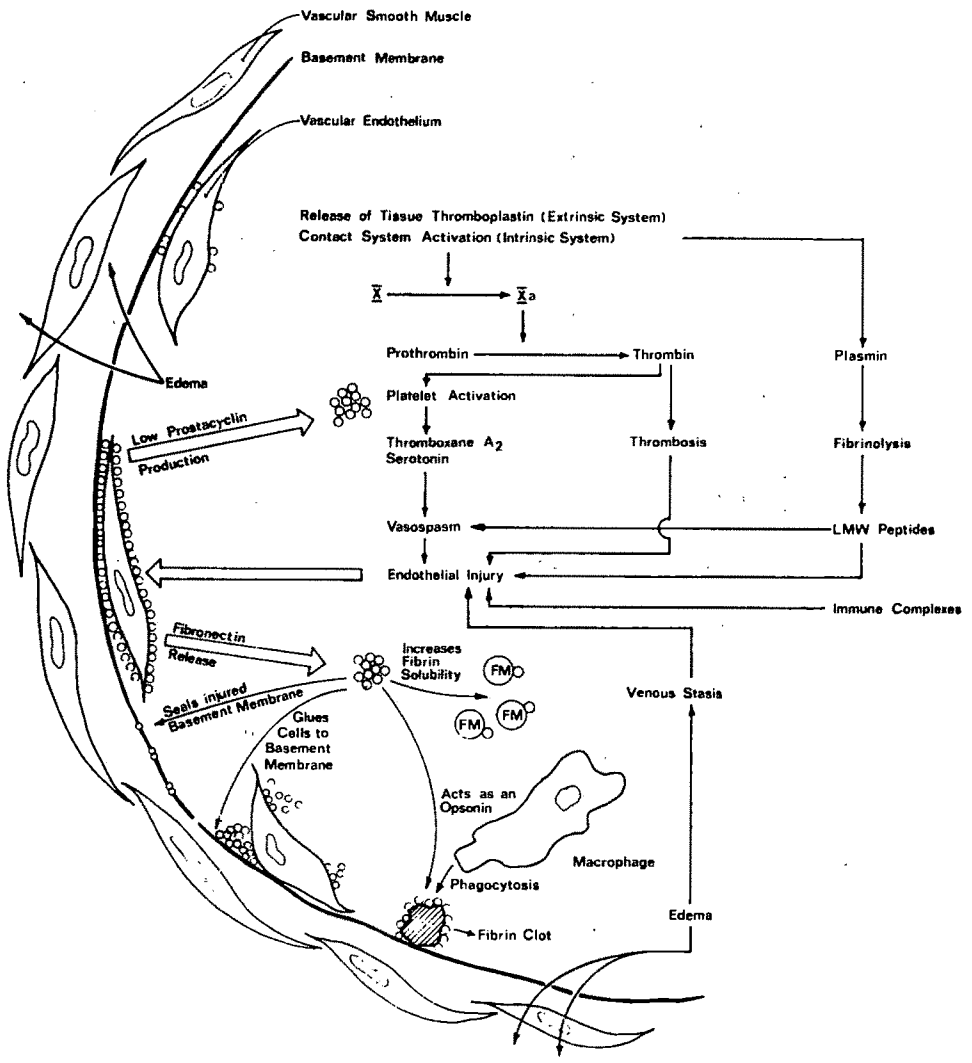


Fig. 1. A model for the effects of pregnancy-induced hypertension (preeclampsia) on the hemostatic system. Note the central role of endothelial injury. *LMW peptides* refers to low molecular weight fibrinolytic peptides; *FM* refers to fibrin monomer.

Results

The clinical characteristics described in Tables IIA and IIB are stratified according to the severity of preeclampsia and the presence of labor at the time of initial coagulation tests. The clinical changes associated with preeclampsia (hypertension, proteinuria, earlier gestational age, and lower birth weight) appeared to be more pronounced among patients recruited for the study before the onset of labor. Consequently, results stratified for preeclampsia and delivery (before or after) are reported separately for patients in labor (Table III) and those not in labor (Table IV).

Statistical analysis of the changes in hemostasis associated with preeclampsia, delivery, and their interaction is presented in Table V; potential confounding factors will be considered later. The interaction terms in this analysis can be interpreted as a measure of how the effect of delivery varies according to the presence and severity of preeclampsia. The overall terms can be interpreted as testing whether preeclampsia, delivery, or their interaction has a global effect on hemostasis, as represented by all of the laboratory parameters. Because all three overall terms were significant, the effects of preeclampsia, delivery, and their interaction on individual laboratory tests are considered in detail.

Preeclampsia was associated with an increase in fibronectin and decreases in antithrombin III and α_2-antiplasmin. Delivery was associated with a decrease in fibronectin and an increase in α_2-antiplasmin. Trends for mean platelet volume and fibrin monomer to decrease after delivery did not attain statistical significance. Decreases in fibronectin after delivery varied significantly according to the presence and severity of preeclampsia. Little change was noted in the normal

group, but progressively larger decreases were noted with mild and severe preeclampsia.

In addition to the summary presented in Table V, statistical evaluation included analysis for confounding variables related to gestational age and recruitment before labor. The parameters of hemostasis were not significantly correlated with gestational age in the 28- to 43-week range of this study ($p > 0.05$ for each parameter). Patients not in labor had similar overall coagulation profiles but had lower antithrombin III ($p < 0.01$), a change limited to preeclamptic patients. No significant interaction of recruitment before labor with preeclampsia or delivery was apparent for either overall coagulation profiles or individual parameters of hemostasis.

Comment

Taken as a whole these findings suggest that vascular endothelial injury plays a central role in the hemostatic changes associated with preeclampsia. When comparing markers of endothelial injury, clotting, and fibrinolysis, fibronectin exhibited the most pronounced changes with preeclampsia. The clinical effect of delivery specific for preeclampsia (i.e., resolution) was associated more closely with fibronectin than the other parameters of hemostasis. It has been reported[11] that pregnancy is normally associated with increases in clotting and fibrinolytic activity and that in preeclampsia there appears to be an exaggeration of this pattern. We are unaware of any previous study that has applied recent advances in coagulation research to compare the roles of endothelial injury, clotting, and fibrinolysis in preeclampsia. We are also unaware of previous research with these techniques to systematically evaluate the usual effects of delivery or the specific effects of delivery for preeclampsia.

When interpreting these results, it is important to remember that blood levels reflect production, metabolism, and/or excretion. Although levels of the new markers are believed to be representative of their roles in hemostasis, other sources of variation could be involved. Fibronectin is produced in the liver and the vascular endothelium. Hepatic impairment or renal excretion could contribute to decreased levels of antithrombin III. Little is known about the metabolism and/or excretion of α_2-antiplasmin. Additional caution is warranted because of variability in the accuracy of the assays. Proteinuria, fibrin monomer, fibrin(ogen) split products, and D-dimer were qualitative or semiquantitative and much less accurate than fibronectin, fibrinogen, antithrombin III, and α_2-antiplasmin. Their qualitative or semiquantitative nature may also have reduced the efficiency of statistical analysis.

Nonetheless, the findings of this study integrate nicely into previously developed concepts of hemostasis (Fig. 1). Preeclampsia is accompanied by endothelial injury, clotting, and fibrinolysis, elements present in DIC. Vascular endothelial injury in preeclampsia could be caused by vasospasm or immune complexes. Endothelial damage may cause decreased prostacyclin production and activation of both clotting and fibrinolysis, resulting in the generation of thrombin and plasmin, respectively. Thrombin consumes antithrombin III and causes intravascular fibrin depositions. Plasmin consumes α_2-antiplasmin and converts fibrin and fibrinogen into fibrin(ogen) split products. Platelet activation leads to release of thromboxane A_2 and serotonin, which, along with low molecular weight fibrinolytic peptides, may induce further vasospasm and/or endothelial injury.[12] Elevated fibronectin may antagonize clotting by enhancing fibrinolysis, aiding in endothelial repair and solubilizing the fibrin monomer, thereby preventing the formation of a fibrin network.[13-14] Fibronectin also acts as an opsonin, allowing phagocytosis of formed blood clots by macrophages from microcirculation.[1] In the absence of clinical signs of DIC, increased fibronectin, accompanied by increased production of platelets and fibrinogen, appears to be adequate to compensate for increased consumption by clotting.

In summary, it appears that vascular endothelial injury plays a central role in the hemostatic changes associated with preeclampsia. This concept is further supported by the observation that it seemed to resolve 24 to 48 hours after delivery. This coincides with the rapid clinical improvement noted in the early puerperium and could contribute to recovery.

REFERENCES

1. Stubbs TM, Lazarchick J, Horger EO. Plasma fibronectin levels in preeclampsia: a possible biochemical marker for vascular endothelial damage. AM J OBSTET GYNECOL 1984;150:885-7.
2. Weiner CP, Brandt J. Plasma antithrombin activity: an aid in the diagnosis of preeclampsia-eclampsia. AM J OBSTET GYNECOL 1982;142:275-81.
3. Weenink GH, Treffers PE, Vijn ME, et al. Plasma antithrombin III levels in preeclampsia. Clin Exp Hypertens 1983;B2:145-62.
4. Gow L, Campbell DM, Ogston D. Blood levels of proteinase inhibitors in preeclampsia. Br J Obstet Gynaecol 1983;90:950-2.
5. López-Llera M, de la Luz Espinosa M, Diaz de León MD, et al. Abnormal coagulation and fibrinolysis in eclampsia. A clinical and laboratory correlation study. AM J OBSTET GYNECOL 1976;124:681-7.
6. Fletcher AP, Alkjaersig NK, Burstein R. The fibrinolytic enzyme function. AM J OBSTET GYNECOL 1979;134:743-51.
7. Pritchard JA, McDonald PC, Gant NF, eds. Williams obstetrics. 17th ed. East Norwalk, Conn.: Appleton-Century-Crofts, 1985:525-8.
8. Mammen EF, Koets MH, Washington BC, et al. Hemostasis changes during cardiovascular bypass surgery. Semin Thromb Hemost 1985;11:281-92.
9. Steele BW, Hills LP, Hellmen RL, et al. Automated fibro-

nectin assay using light scattering optics on a centrifugal analyzer. Clin Biochem 1984;17:157-61.
10. Bick RL. Disseminated intravascular coagulation and related syndromes: etiology, pathophysiology, diagnosis, and management. Am J Hematol 1978;5:265-82.
11. McKay DG. Chronic intravascular coagulation in normal pregnancy and preeclampsia. Contrib Nephrol 1982;25: 108-19.
12. Busch C, Gerdin B. Effect of low molecular weight fibrin degradation products on endothelial cells in culture. Thromb Res 1981;22:33-9.
13. Mosher DF. Physiology of fibronectin. Annu Rev Med 1984;35:561-75.
14. Kaplan JE. Plasma fibronectin and resistance to thrombosis during sepsis. Adv Shock Res 1984;7:159-72.

Elevated maternal serum α-fetoprotein levels and oligohydramnios: Poor prognosis for pregnancy outcome

Susanna N. Dyer, M.S.N., Barbara K. Burton, M.D., and Lewis H. Nelson, M.D.
Winston-Salem, North Carolina

The outcome of 21 pregnancies with elevated maternal serum α-fetoprotein levels associated with oligohydramnios was studied. Seven of the 21 pregnancies ended in spontaneous abortion or intrauterine fetal death before week 24 of pregnancy. Five patients experienced premature labor between 24 and 26 weeks of gestation; each fetus was either stillborn or died in the immediate neonatal period. Four patients were delivered of infants after 32 weeks of gestation; each infant was either stillborn or died in the immediate neonatal period. Four patients electively had their pregnancies terminated. One patient was delivered at term of a healthy, growth retarded male infant who on follow-up at age 17 months was developmentally normal. Only three cases were associated with a fetal defect. Patients should be counseled that, even in the absence of a demonstrable cause for diminished amniotic fluid on ultrasonography, raised maternal serum α-fetoprotein levels coupled with oligohydramnios seem to carry a poor prognosis. (AM J OBSTET GYNECOL 1987;157:336-9.)

Key words: Maternal serum α-fetoprotein, oligohydramnios

The association between raised concentrations of maternal serum α-fetoprotein and open fetal neural tube defects has been well documented.[1-3] Because more than 90% of all affected infants are born to women with no previous family history of a neural tube defect, the majority can be detected prenatally only by a testing program available to all pregnant women. An increasing number of women are being offered maternal serum α-fetoprotein screening as facilities capable of performing the test become widespread. It is now becoming apparent that elevated maternal serum α-fetoprotein levels even in the absence of a neural tube defect may indicate that a pregnancy is at high risk, and otherwise unexplained maternal serum α-fetoprotein elevations have been associated with an increased risk of spontaneous abortion and stillbirth,[1,4] low birthweight,[5,6] and other congenital anomalies.[7]

Previous reports have linked elevated maternal serum α-fetoprotein levels to second-trimester oligohydramnios. We describe a series of 21 patients found through diagnostic testing to have oligohydramnios; the tests were done after elevated maternal serum α-fetoprotein levels were discovered. Pregnancy outcomes for each case are discussed.

Methods

All patients studied were participants in the Screening Program for Neural Tube Defects administered by the Department of Pediatrics at the Bowman Gray School of Medicine, Winston-Salem, North Carolina. Initial maternal serum α-fetoprotein screening was generally performed at a gestational age of 16 to 18 weeks. Levels of ≥2.5 times the median were considered elevated, and further evaluation was rec-

From the Section on Medical Genetics, Department of Pediatrics, and the Department of Obstetrics and Gynecology, Bowman Gray School of Medicine, Wake Forest University.
The Screening Program was funded by the North Carolina Department of Human Resources, Health Services Division, Contract No. 6210246.
Received for publication October 13, 1986; accepted March 19, 1987.
Reprint requests: Susanna N. Dyer, Neural Tube Defects Screening Program, Department of Pediatrics, Bowman Gray School of Medicine, Wake Forest University, 300 S. Hawthorne Road, Winston-Salem, NC 27103.

Table I. Summary of patients with elevated maternal serum α-fetoprotein and oligohydramnios

Patient No.	*MSAFP MOM**	*Additional ultrasound findings*	*Pregnancy outcome*	*Autopsy results*	*Comments*
1	3.3/3.8		EPT	Normal	
2	8.5/10.0	IUGR	IUFD at 20 wk	Multiple placental infarcts	
3	3.0/4.0		IUFD at 20 wk	Amnionitis	
4	7.0/5.5	Fetal contractures	Spontaneous abortion at 20 wk	Multiple contractures	
5	2.8		IUFD at 24 wk	Normal	
6	9.4/12.0	Encephalocele	Stillborn at 32 wk	Not performed	Encephalocele, polydactyly, ambiguous genitalia on clinical examination
7	5.2/4.3		Premature delivery at 24 wk, died	Normal	
8	5.0/4.5		Spontaneous abortion at 20 wk	Not performed	No defects on clinical examination
9	5.1/4.5		EPT	Normal	
10	2.9/2.7		IUFD at 19 wk	Normal	
11	3.8/2.8	IUGR	Spontaneous abortion at 23 wk	Normal	
12	3.9/3.0	Kidney, bladder not visualized	Premature delivery at 32 wk, died	Not performed	Potter's syndrome on clinical examination
13	4.2/2.8	IUGR, encephalocele	Delivery at 40 wk, died	Encephalocele	Class A diabetes
14	2.5/3.8		Premature delivery at 25 wk, died	Not performed	No defects on clinical examination
15	2.8/2.6	FHR decelerations	Premature delivery at 37 wk, IUGR	Not performed	Doing well at age 17 mo
16	2.6/2.4		EPT	Normal	
17	4.0/5.3		Premature delivery at 24 wk, died	Not performed	No defects on clinical examination
18	2.5/2.0		Premature delivery at 26 wk, died	Not performed	No defects on clinical examination
19	3.6/5.1	Enlarged kidney and bladder	EPT	Posterior urethral valves, hydronephrosis	Potter facies
20	3.9		Spontaneous abortion at 22 wk	Not performed	No defects on clinical examination
21	3.6		Premature delivery at 32 wk, died	Not performed	Multiple contractures

MSAFP MOM = Maternal serum α-fetoprotein multiples of the median; EPT = elective pregnancy termination; IUGR = intrauterine growth retardation; IUFD = intrauterine fetal death; FHR = fetal heart rate.

*If patient had two samples assayed, both values are given.

ommended. Patients with an initial maternal serum α-fetoprotein elevation usually had a second sample quantitated at least 1 week later. After two consecutive elevations, women became candidates for ultrasound examination and possibly amniocentesis. Before these procedures, each patient received genetic counseling. Pregnancy outcome data were collected from delivery records or physician's offices on each patient who was considered a candidate for further testing. From June 1, 1978 to December 31, 1985, 34,932 patients participated in the screening program. Of these, 794 (2.3%) patients had elevated maternal serum α-fetoprotein levels on two separate occasions. As part of the screening protocol, each patient was recommended for consultative ultrasound to rule out a neural tube defect or other fetal defect. Of these, 21 patients were found to have oligohydramnios as defined by absence of amniotic fluid pockets >1 cm in depth. Ultrasound findings, pregnancy outcome, and autopsy data were reviewed for each case.

Results

Twenty-one cases of elevated maternal serum α-fetoprotein levels associated with oligohydramnios were identified during diagnostic testing after the discovery of elevated maternal serum α-fetoprotein levels. The findings are summarized in Table I. Of these, only one resulted in the birth of a healthy, viable infant. Seven pregnancies ended in spontaneous abortion or intrauterine fetal demise before 24 weeks of gestation.

Five pregnancies ended in premature labor and delivery between 24 to 26 weeks of gestation; each fetus was either stillborn or died in the immediate neonatal period. Four patients were delivered after 32 weeks of gestation; each infant was either stillborn or died in the immediate neonatal period. Four patients electively had their pregnancies terminated on the basis of counseling regarding the prognostic significance of oligohydramnios coupled with raised maternal serum α-fetoprotein levels. Autopsy results were available for 12 of the 21 patients, of which only three revealed fetal defects, including two cases of encephalocele and one case of urinary tract anomaly. Renal agenesis was suspected in another case; however, autopsy was not performed. An additional two fetuses had multiple joint contractures, presumably caused by intrauterine compression.

Case number 15 represents the only pregnancy in the series that resulted in the birth of a viable infant. Emergency cesarean section was performed at 37 weeks of gestation for acute preeclampsia and fetal heart rate decelerations. A 2000 gram male infant was delivered with a 1-minute Apgar score of 6 and a 5-minute Apgar score of 9. Hospitalization was complicated only by an elevated bilirubin level that spontaneously resolved. Aside from intrauterine growth retardation, no other anomalies were noted on physical examination. The infant was discharged after 10 days. On follow-up at age 17 months, he weighed 23 pounds (tenth percentile) and was developmentally normal.

Amniotic fluid levels of α-fetoprotein were available in four cases. Cases 1 and 15 had amniotic fluid α-fetoprotein levels below the mean, case 17 was slightly above the mean but within 2 SD, and case 2 was >5 SD above the mean. Karyotyping demonstrated a normal chromosome complement on all four samples of amniotic fluid.

Comment

Elevated levels of maternal serum α-fetoprotein have been associated with open fetal neural tube defects and other congenital anomalies, spontaneous abortion, fetal death, stillbirth, and low birth weight. Therefore, even in the absence of a neural tube defect, raised concentrations of maternal serum α-fetoprotein may serve as a predictor of a high-risk pregnancy.

Four previous reports have described a total of 18 cases of second-trimester oligohydramnios associated with elevated maternal serum α-fetoprotein levels. Balfour and Laurence[8] reported two infants who had renal agenesis, which was detected on postmortem examination. Both infants were delivered at 34 weeks of gestation and died in the immediate neonatal period. Seller and Child[9] described four cases, one of which resulted in the birth of an infant who was described as alive and progressing. Two patients chose to have their pregnancies terminated. No fetal defects were present in either fetus except for compression deformities observed in one. One patient prematurely was delivered of a male infant at 29 weeks of gestation with joint contractures, who died in the immediate neonatal period. Autopsy was not performed. Stirrat et al.[10] identified six cases, five of which ended in intrauterine or neonatal death. Of these, two were associated with extrauterine pregnancy, one with chronic leakage of amniotic fluid, and one with possible renal agenesis. One patient was delivered of a normal female infant at term. Koontz et al.[11] reported six additional cases, four of which were associated with urinary tract abnormalities. Five patients elected to have their pregnancies terminated; the sixth experienced intrauterine fetal death.

The present series brings to a total of 39 the reported cases of oligohydramnios associated with elevated maternal serum α-fetoprotein levels. Of these, four cases of renal agenesis have been identified, three cases of urinary tract obstruction, two cases of extrauterine pregnancy, and one case of chronic leakage of amniotic fluid. For these cases the cause of oligohydramnios is clear. For the remaining 29 cases (74%) the cause is unknown. Twenty-five of the 39 pregnancies (64%) ended in spontaneous abortion, intrauterine fetal death, stillbirth, or neonatal death. Eleven patients (28%) chose to terminate their pregnancies. Only three pregnancies (8%) have been associated with the birth of a viable infant.

It appears that in some cases oligohydramnios in itself is sufficient to explain elevated maternal serum α-fetoprotein levels. However, the relationship between oligohydramnios and elevated maternal serum α-fetoprotein levels is unclear. The mechanism by which open neural tube defects and other congenital anomalies elevate maternal serum α-fetoprotein levels has been reviewed by Brock[12] and appears to be linked to elevated levels of amniotic fluid α-fetoprotein. Increased amniotic fluid α-fetoprotein may occur when any open lesion such as a neural tube defect, ventral wall defect, or sacrococcygeal teratoma allows increased diffusion of α-fetoprotein from the fetal cerebrospinal fluid or serum. Another known cause of elevated maternal serum α-fetoprotein levels, congenital nephrosis of the Finnish type, produces fetal proteinuria with subsequent increased amniotic fluid α-fetoprotein. Finally, elevated amniotic fluid α-fetoprotein may be produced by altered amniotic fluid protein turnover from decreased fetal swallowing, as in duodenal or esophageal obstruction.

In contrast, it does not appear that elevated maternal serum α-fetoprotein levels found in pregnancies affected with oligohydramnios can be linked to elevated amniotic fluid α-fetoprotein, since in three of the four

cases in our series in which amniotic fluid α-fetoprotein was measured, this level was normal. Because it appears that urination is the principle route by which α-fetoprotein normally reaches amniotic fluid, one might expect urinary tract anomalies to result in low levels of amniotic fluid α-fetoprotein. Therefore, for the eight reported cases of urinary tract anomalies, elevated maternal serum α-fetoprotein levels may appear to be paradoxical. Amniotic fluid measurements were not available for any of these cases. Koontz et al.[11] speculated that elevated fetal serum α-fetoprotein levels may result from the inability of the fetus to excrete α-fetoprotein when urinary tract anomalies are present. Consequently, elevated levels of maternal serum α-fetoprotein may result from the greatly increased fetal-maternal concentration gradient across the placental membrane. For the other cases of oligohydramnios, adverse events might occur early in pregnancy, which simultaneously result in oligohydramnios and elevation of maternal serum α-fetoprotein levels, perhaps through some alteration of placental structure or function. Therefore, although no causal relationship may be present between oligohydramnios and elevated maternal serum α-fetoprotein levels, a common origin may exist for both.

Patients should be counseled that elevated maternal serum α-fetoprotein levels coupled with oligohydramnios appear to carry a poor prognosis for the pregnancy. Even in the absence of a demonstrable fetal defect on ultrasonography, the likelihood of a normal outcome remains low.

We gratefully acknowledge the support and efforts of the participating physicians in western North Carolina, without whom this project would not have been possible.

REFERENCES

1. Burton BK, Sowers SG, Nelson LH. Maternal serum α-fetoprotein screening in North Carolina: experience with more than twelve thousand pregnancies. Am J Obstet Gynecol 1983;146:439-44.
2. Macri JN, Weiss RR. Prenatal serum α-fetoprotein screening for neural tube defects. Obstet Gynecol 1982;59: 633-9.
3. Milunsky A, Alpert E, Neff RK, et al. Prenatal diagnosis of neural tube defects: IV. Maternal serum alpha-fetoprotein screening. Obstet Gynecol 1980;55:60-6.
4. Wald N, Cuckle H, Stirrat GM, et al. Maternal serum alpha-fetoprotein and low birth weight. Lancet 1977;2: 268-70.
5. Wald NJ, Cuckle HS, Borehum J, et al. Maternal serum alpha-fetoprotein and low birth weight. Br J Obstet Gynaecol 1980;87:860-3.
6. Brock DJH, Barron L, Raab GM. The potential of midtrimester maternal plasma alpha-fetoprotein measurement in predicting infants of low birth weight. Br J Obstet Gynaecol 1980;87:582-5.
7. Burton BK, Dillard RG. Outcome in infants born to mothers with unexplained elevations of maternal serum α-fetoprotein. Pediatrics 1986;77:582-6.
8. Balfour RP, Laurence KM. Raised serum AFP levels and fetal renal agenesis. Lancet 1980;1:317.
9. Seller MJ, Child AH. Raised maternal serum alpha-fetoprotein, oligohydramnios, and the fetus. Lancet 1980;1:317-8.
10. Stirrat GM, Gough JD, Bullock S. Raised maternal serum AFP, oligohydramnios, and poor fetal outcome. Br J Obstet Gynaecol 1981;88:231-5.
11. Koontz WL, Seeds JW, Adams NJ, et al. Elevated maternal serum alpha-fetoprotein, second trimester oligohydramnios, and pregnancy outcome. Obstet Gynecol 1983;62: 301-4.
12. Brock DM. Mechanisms by which amniotic-fluid alpha-fetoprotein may be increased in fetal abnormalities. Lancet 1976;2:345-6.

Mycotic aneurysms in pregnancy

Guy M. Boike, M.D., Norman Gove, M.D., Mitchell P. Dombrowski, M.D., Gunter Deppe, M.D., and Robert J. Sokol, M.D.
Detroit, Michigan

The presentation, management, and outcome of three cases of femoral artery mycotic aneurysm in pregnancy are described. All resulted from illicit intravenous drug use. While the literature dealing with drug abuse in pregnancy is large, this specific problem has received little attention. Comparison of these cases to a large reported series of nonpregnant patients with mycotic aneurysms from our institution demonstrates that pregnancy does not alter the natural course of this condition. The presence of a mycotic aneurysm in pregnancy does, however, appear to lead to significant maternal and fetal morbidity. Guidelines for diagnosis and management are discussed. (AM J OBSTET GYNECOL 1987;157:340-1.)

Key words: Pregnancy, aneurysms, intravenous drug abuse

Arterial aneurysms are an infrequent complication of pregnancy. The term mycotic aneurysm was first coined by Osler in 1885 to describe aortic aneurysms resembling "fungal growths" in a man with endocarditis. The potentially catastrophic consequences of aneurysmal rupture and the accompanying infectious morbidity emphasize the need for early diagnosis and appropriate management. The purpose of this report is to communicate our experience with three intravenous drug abusers whose pregnancies were complicated by femoral artery mycotic aneurysms.

Case reports

Case 1. A 33-year-old woman, gravida 2, para 1, abortions 0, was admitted at 20 weeks' gestation for bleeding from an injection site in the groin. A pulsatile right groin mass with a bruit was present and an arteriogram demonstrated a 2 cm aneurysm of the deep femoral artery. Ligation of the superficial, common, and deep femoral artery was performed. Cultures from the aneurysm grew nafcillin-resistant *Staphylococcus aureus* and *Klebsiella pneumoniae*. Estimated operative blood loss was 700 ml and a transfusion of 2 units of blood was required. At 25 weeks' gestation, ischemic changes and purulent drainage from the wound necessitated a right above-the-knee amputation. The patient remained hospitalized without further complications. At term, an elective induction of labor resulted in the vaginal delivery of a healthy, appropriate for gestational age infant. After an uncomplicated postpartum course, both mother and infant were discharged.

From the Department of Obstetrics and Gynecology, Hutzel Hospital/Wayne State University.
Received for publication October 3, 1986; revised February 18, 1987; accepted March 12, 1987.
Reprint requests: Guy M. Boike, M.D., Hutzel Hospital, Department of Obstetrics and Gynecology, 4707 St. Antoine, Detroit, MI 48201.

Case 2. A 34-year-old woman, gravida 6, para 3, abortions 2, was admitted at 34 weeks' gestation with a draining right groin abscess and a swollen, painful left thigh. A pulsatile left groin mass with a bruit was present and an arteriogram confirmed a 4 cm left femoral aneurysm. The draining right groin abscess grew nafcillin-resistant *S. aureus*. Shortly after admission, the patient experienced spontaneous rupture of the membranes accompanied by labor and was delivered vaginally of a preterm, small for gestational age infant that experienced mild respiratory distress. After delivery the patient underwent ligation of the left common and superficial femoral arteries without complication. The patient remained hospitalized for 25 days and was maintained on intravenous vancomycin while the wound healed.

Case 3. A 34-year-old woman, gravida 3, para 2, was admitted at 33 weeks' gestation with a swollen, painful left thigh. The patient stated that a pulsatile left groin mass had been present for 1 year. An arteriogram revealed a 9.5 cm left femoral aneurysm. In an effort to avoid operation during pregnancy and because of the relative proximity to term, the patient was managed conservatively while awaiting fetal maturity. At term, an elective cesarean delivery was performed because of a breech-vertex twin presentation and to avoid the risk of aneurysmal rupture during labor. The patient was delivered of otherwise healthy discordant twins (twin A, appropriate for gestational age; twin B, small for gestational age). After delivery the patient did well but refused ligation of the aneurysm and signed out against medical advice on the fifth postoperative day.

Comment

Care of the pregnant patient who engages in intravenous drug abuse is complicated by many obstetric and medical problems. All of our patients received no prenatal care, had uncertain gestational dates, and experienced complications from intravenous drug abuse. Previous reports of mycotic aneurysms in pregnancy are limited, possibly reflecting underreporting. Dir-

ghani and Ansari[1] reported a case of a ruptured right external iliac artery aneurysm secondary to drug abuse in a pregnant patient. This led to an intrauterine fetal death and significant maternal morbidity.

A series of 50 nonpregnant patients with mycotic aneurysms has been reported from our institution.[2] Local pain, swelling, bleeding, and symptoms of vascular insufficiency were the most common presenting complaints. A pulsatile, indurated mass with an associated bruit was present in nearly all cases, with the femoral artery being the most frequently involved site. Approximately 80% of the patients were adequately treated with simple ligation and excision, but the remaining 20% required bypass or amputation. Resistant *S. aureus* was isolated from more than three quarters of the cases.

A thorough history and physical examination are necessary in patients who abuse drugs intravenously, with special attention given to complaints referred to injection sites and extremities. Careful palpation for fluctuance or pulsations and auscultation for bruits is essential for the diagnosis of an underlying abscess or aneurysm. Arteriograms should be liberally used for confirmation. Operation for this condition during pregnancy presents potential fetal risks including anesthetic complications, hypotension from supine positioning, and decreased placental perfusion from operative blood loss. The delay in surgical treatment may result in aneurysmal rupture with maternal hypotension, decreased placental perfusion, and possibly fetal death.[1] The effect of second-stage valsalva maneuvers may increase the risk of aneurysmal rupture, although there is little evidence to confirm this. At all gestational ages, the risk of operation to the undelivered fetus must be weighed against delaying definitive treatment.

It is difficult to formulate a management scheme on the basis of this limited experience; however, the following guidelines would appear appropriate: (1) Leaking or expanding aneurysms require immediate operation irrespective of gestational age. If viable, the fetus should be monitored during operation and the attending obstetrician prepared to perform a cesarean delivery for fetal indications. (2) Aneurysms diagnosed during the first and second trimesters should be surgically treated. (3) Near term, attempts at conservative management awaiting fetal maturity should be considered, with delivery prior to operation. (4) Careful attention to bacterial isolates and resistance patterns is necessary to optimize antibiotic therapy. With early diagnosis and intensive surgical-obstetric management, the excessive morbidity of this condition in pregnancy may be minimized.

We wish to acknowledge the expert technical assistance of Suellen J. Pence.

REFERENCES

1. Dirghani JK, Ansari MR. Rupture of mycotic aneurysm during pregnancy: another hazard of drug abuse. Henry Ford Hosp J 1982;30:93.
2. Johnson JR, Ledgerwood AM, Lucas CE. Mycotic aneurysm: new concepts in therapy. Arch Surg 1983;118:577.

A screening test (GBS-Test) for urogenital carriage of group B streptococci

Karen Christensen, M.D., Ph.D., and Poul Christensen, M.D., Ph.D.
Lund, Sweden

A commercial kit for the detection of group B streptococci (GBS-Test) is described. The test is based on orange pigment production after 2 days' incubation at 37° C. It showed a 100% specificity and a sensitivity superior to that of a conventional selective broth method. (Am J Obstet Gynecol 1987;157:341-2.)

Key words: Group B streptococci, pregnancy, screening

From the Fdeon Science Park.
Received for publication July 22, 1986; revised February 4, 1987; accepted March 12, 1987.
Reprint requests: Dr. Karen K. Christensen, Fdeon Science Park, S-22370 Lund, Sweden.

Recently, a successful prevention program for the majority of early-onset group B streptococcal infections was described, based on screening during pregnancy.[1] The present paper describes a new commercial kit

Table I. Comparison of a conventional selective method and the GBS-Test for detection of group B streptococci in the urethra, cervix, and rectum

Site of collection of specimen	*No. of specimens containing group B streptococci per No. collected*	*Conventional selective method*		*GBS-Test*			
		No. of tests positive for group B streptococci	*Sensitivity (%)*	*No. of tests positive*		*Sensitivity (%)*	
				Day 1	*Day 2*	*Day 1*	*Day 2*
Urethra	58/200	53	91	57	58	98	100
Cervix	53/200	46	87	48	49	91	92
Rectum	29/100	25	86	21	25	72	86
All specimens	140/500	124	89	126	132	90	94

(GBS-Test*) which is suitable for the detection of group B streptococci during pregnancy.

The test consists of a tube containing 1.5 ml of broth. Each specimen to be investigated for group B streptococci was obtained with a cotton-tipped swab, which was then whisked out in the broth. The tube was capped and incubated for 2 days at 37° C. On days 1 and 2, tubes were inspected for orange pigmentation, indicating the presence of group B streptococci.

With this technique, 500 urogenital specimens were evaluated, 200 from the urethra, 200 from the cervix, and 100 from the rectum. All specimens were transported in Stuart medium within 24 hours of collection. Each specimen was whisked out in 1 ml of Todd-Hewitt broth at a temperature of 4° to 8° C. Two sterile cotton swabs were then dipped simultaneously in the broth; one of them was used for selective broth medium isolation of group B streptococci, and the other was used for detection of these bacteria with the GBS-Test.

When a GBS-Test was positive but no β-hemolytic streptococci had been detected on the blood agar plate, new attempts were made to isolate the bacteria from the Todd-Hewitt broth, by use of the selective method. The results of the "second look" were not included in the data given for the selective method (Table I).

The sensitivity of the conventional method was 91% for the urethra, 87% for the cervix, and 86% for the rectum (Table I). The respective figures for the GBS-Test were 100%, 92%, and 86% after 2 days of incubation. No false positive results were recorded with the GBS-Test (specificity = 100%).

All of 14 group B streptococcal strains isolated from cerebrospinal fluid or blood of newborn infants gave positive results with the GBS-Test, 13 after 24 hours' incubation and the fourteenth strain after 42 hours' incubation.

The data thus demonstrated that the GBS-Test was at least as reliable as a conventional selective method used for detection of group B streptococci at bacteriologic laboratories.

Although it does not appear to be quicker than conventional methods, the test permits diagnosis of group B streptococci at the doctor's office. The simple handling and easy reading of the test and a cost below 6 dollars for two tubes (that is, one screening occasion) has radically changed the cost-benefit ratio in favor of routine screening for group B streptococci during pregnancy. When this test is combined with prophylactic antibiotics in selected patients at risk,[1] we now have access to an instrument that can prevent a substantial part of early-onset group B streptococcal infections in newborn infants.

*Patent pending.

REFERENCE

1. Boyer KM, Gotoff SP. Prevention of early-onset neonatal group B streptococcal disease with selective intrapartum chemoprophylaxis. N Engl J Med 1986;314:1665-9.

Fetal growth retardation as a complication of pregnancy in patients with neurofibromatosis

Isaac Blickstein, M.D., and Moshe Lancet, M.D.
Rehovot, Israel

Neurofibromatosis adversely affects reproductive performance. Fetal growth retardation as a complication of pregnancy in patients with neurofibromatosis had not been widely documented. The present cases suggest that pregnant patients with neurofibromatosis may have an increased risk for growth-retarded babies. (AM J OBSTET GYNECOL 1987;157:343.)

Key words: Neurofibromatosis, pregnancy complications, fetal growth retardation

Von Recklinghausen's neurofibromatosis is associated with many pregnancy complications. The pregnant state may, in turn, aggravate the disease to the point of life-threatening situations. Belton et al.[1] reported what they assumed to be the first case of intrauterine growth retardation in association with neurofibromatosis in pregnancy. The present cases document five additional growth-retarded babies born to two patients with neurofibromatosis.

Case reports

Case 1. S. A., a 30-year-old gravida 2, para 1, was admitted at 38 weeks' gestation in active labor. Mild neurofibromatosis was diagnosed at her previous delivery in 1984 by the attending obstetrician. Both pregnancies were achieved after ovulation induction with clomiphene, human menopausal gonadotropin and human chorionic gonadotropin, and bromocriptine. They were unremarkable with respect to weight gain, hemoglobin levels, and blood pressure. The first baby was growth retarded (2400 gm at 39 weeks' gestation) and had inherited his mother's disease. The second one was born at 38 weeks' gestation and was severely growth retarded (1820 gm). The placentas were with no stigmata of neurofibromatosis, relatively small, with areas of infarction.

Case 2. I. R., a 23-year-old gravida 3, para 2, was admitted at 35 weeks' gestation. She had mild neurofibromatosis since childhood. She previously had a 1500 gm growth-retarded baby at 35 weeks and a second one at 39 weeks weighing 2250 gm.

Her three pregnancies were unremarkable with respect to the aforementioned factors. The third pregnancy ended by a cesarean section because of preterm rupture of the membranes and fetal distress. A 1850 gm growth-retarded baby was born. The placenta was relatively small but otherwise normal. The two older children do not have signs of neurofibromatosis, nor have any such signs been found in the newborn infant.

Comment

Possible explanations for intrauterine growth retardation associated with neurofibromatosis include: (1) Vascular changes characteristic of neurofibromatosis may lead to insufficient blood supply to the gravid uterus, (2) a rapidly growing neurofibromatic pelvic tumor may displace the uterus and compress the major blood vessels, and (3) nontoxemic hypertension is thought to be an invariable feature of pregnant patients with neurofibromatosis.

Belton et al.[1] reported a case of fetal growth retardation and oligohydramnios in association with neurofibromatosis. What they claimed as the first reported case had been criticized as coincidental.[2] Our cases suggest that fetal growth might be impaired by the disease process of neurofibromatosis. Since we could not single out other causes for intrauterine growth retardation in our patients, neurofibromatosis and its adverse effects on the uteroplacental unit seem to be most likely.

Neurofibromatosis affects many organs and systems, and thus a multifactorial etiology for intrauterine growth retardation seems probable. Further observations may disclose the actual magnitude of this complication in the relatively infrequent combination of pregnancy and neurofibromatosis.

From the Department of Obstetrics and Gynecology, Kaplan Hospital (affiliated with Hadassah-Hebrew University School of Medicine).
Received for publication November 13, 1986; accepted December 30, 1986.
Reprint requests: Isaac Blickstein, M.D., Department of Obstetrics and Gynecology, Kaplan Hospital, 76-100 Rehovot, Israel.

REFERENCES

1. Belton SR, Ferguson JE, Catanzarite VA. Neurofibromatosis and pregnancy: report of a case complicated by intrauterine growth retardation and oligohydramnios. AM J OBSTET GYNECOL 1984;149:468-9.
2. Davies BR. Neurofibromatosis: cause or coincidence of pregnancy complications? [Letter.] AM J OBSTET GYNECOL 1985;152:914.

γ-Glutamyl transferase and mean cell volume reveal maternal alcohol abuse and fetal alcohol effects

Olavi Ylikorkala, M.D., Ph.D., Ulf-Håkan Stenman, M.D., Ph.D., and Erja Halmesmäki, M.D.

Helsinki, Finland

Serial measurements of γ-glutamyl transferase and mean cell volume were done in 85 pregnant alcohol abusers (320 samples). These included 26 moderate drinkers, 30 heavy drinkers, and 29 alcoholic women. Studies of 28 pregnant women who denied the use of alcohol (138 samples) showed that pregnancy itself had no effect on γ-glutamyl transferase and mean cell volume. However, γ-glutamyl transferase was increased in 14.9%, 31.7% and 59.1% of the samples collected from moderate drinkers, heavy drinkers, and alcoholic women, respectively, and likewise mean cell volume was elevated in 10.8%, 17.2%, and 40.2%, respectively. Either γ-glutamyl transferase or mean cell volume was high or both were high in 27.1% of the moderate drinkers, in 38.3% of the heavy drinkers, and in 76.3% of the alcoholic women. Fetal alcohol effects developed in 42 cases. Increased γ-glutamyl transferase predicted fetal alcohol effects in 61.5%, with a relative risk of 2.51. Elevated mean cell volume predicted fetal alcohol effects in 40.9%, with a relative risk of 2.40. These results suggest that the assay of γ-glutamyl transferase and mean cell volume can be valuable in monitoring pregnant women with recognized or suspected alcohol abuse. (AM J OBSTET GYNECOL 1987;157:344-8.)

Key words: Alcohol abuse in pregnancy, fetal alcohol effects, γ-glutamyl transferase, mean cell volume

The measurements of serum γ-glutamyl transferase activity and mean cell volume are the best established tests for the detection of alcohol abuse in women who are not pregnant.[1-6] Excessive alcohol consumed during pregnancy is one of the most common teratogens known today.[7, 8] This may be one reason pregnant women often are most reluctant to admit their actual alcohol consumption.

Objective biochemical tests are therefore needed to reveal alcohol use during pregnancy. Because pregnancy may affect the responses of these markers to alcohol intake, data on γ-glutamyl transferase and mean cell volume in women who use alcohol but are not pregnant[1-6] cannot be interpolated to pregnant subjects. Therefore we assessed the value of γ-glutamyl transferase and mean cell volume in the detection of alcohol abuse and fetal alcohol effects during pregnancy.

From the Departments of Obstetrics and Gynecology, University of Helsinki.

Supported by the Finnish Academy of Science and Alcohol Research Foundation.

Received for publication December 11, 1986; revised April 3, 1987; accepted April 27, 1987.

Reprint requests: Olavi Ylikorkala, M.D., Department of Obstetrics and Gynecology, University of Helsinki, Haartmaninkatu 2, 00290 Helsinki, Finland.

Material and methods

From April 1983, staff members of antenatal clinics in Helsinki and its suburbs were trained to recognize alcohol abuse (history of previous use, social reports, previous treatments, drunkenness at follow-up). To the end of 1985, 85 patients with recognized alcohol problems had been identified. They were admitted to a special outpatient university clinic for pregnant problem drinkers. The patients were interviewed about their alcohol use at registration and were counseled to refrain from alcohol use or, if that was impossible, to limit its use as much as possible. The patients were scheduled to visit the department at 2- to 4-week intervals, when a detailed history on alcohol use since the preceding visit was taken and further counseling and support were given. The patients were advised to use iron supplements, but no folic acid supplementation was used.

Of 85 patients, 29 women has consumed two to 20 "drinks" daily during early pregnancy. They were regarded as alcoholic because their history of alcohol abuse was long (up to 20 years) and they had several alcohol-related social problems. Thirty women who had one to 10 "drinks" daily were classified as heavy drinkers because, although they drank heavily, they lacked other features typical for alcoholism. Twenty-six women consumed alcohol periodically (mostly during weekends), up to 10 "drinks" daily, and they were clas-

Table I. Clinical characteristics of the study populations (mean ± SD)

	Moderate drinkers	*Heavy drinkers*	*Alcoholic women*	*Control women*
No. of women	26	30	29	28
No. of GGT assays	107	120	93	135
No. of MCV assays	102	122	97	138
Age (yr)	27.0 ± 7.8	28.1 ± 6.0	29.2 ± 4.3	27.3 ± 5.1
Smoking	21 (82%)	25 (82%)	21 (71%)	6 (21%)
Infants with fetal alcohol effects	1	13	28	—

GGT = γ-Glutamyl transferase; MCV = mean cell volume.

Table II. Sensitivities, predictive values, and relative risks of increased γ-glutamyl transferase and mean cell volume in revealing alcohol abuse during pregnancy

	Moderate drinkers	*Heavy drinkers*	*Alcoholic women*	*All drinkers*
γ-Glutamyl transferase				
Sensitivity (%)	14.9	31.7†	59.1*	34.1*
Predictive value (%)	69.5	84.4	88.7	93.4
Relative risk	1.67	2.16	3.87	1.51
Mean cell volume				
Sensitivity (%)	10.8	17.2†	40.2*	22.1*
Predictive value (%)	64.7	77.8	84.8	91.0
Relative risk	1.59	1.79	2.83	1.41

*Comparison between γ-glutamyl transferase and mean cell volume: $p < 0.01$.
†Comparison between γ-glutamyl transferase and mean cell volume: $p < 0.001$.

sified as moderate drinkers (Table I). Sixty-six patients (78%) also smoked. Otherwise the women were apparently healthy and had no evidence of liver disease. Fifty-five patients (65%) reported that they had reduced their alcohol consumption since their registration (Halmesmäki E, unpublished data).

Infants born to these 85 women were thoroughly studied at ages 5 days (n = 85), 4 months (n = 72), 6 months (n = 72), and 12 months (n = 47) by pediatricians. The diagnosis of fetal alcohol effects required one of the following criteria: growth retardation (less than the tenth normal percentile); distinctive facial characteristics (low nasal bridge, short nose, short palpebral fissures, indistinct philtrum, thin upper lip); neurological aberrations; and developmental delay. Twenty-two such infants were born. In addition, 20 infants fulfilled three of the criteria (growth retardation, distinctive facial features, neurological aberrations), and they were regarded as suffering from a complete "fetal alcohol syndrome."[7, 8] Thus alcohol-induced fetal damage was observed in 42 of 85 infants (49%).

Twenty-eight healthy women who denied the use of alcohol had follow-up throughout pregnancy as a control group (Table I). They all were advised to use iron supplementation starting at 12 to 16 weeks of pregnancy, but no folic acid was prescribed. Six of them (21%) smoked. The pregnancies were uneventful, and the infants were healthy.

Venous blood samples collected at each visit from the patients (on average 3.7 samples per patient) and controls (on average 4.8 samples per control) were assayed for γ-glutamyl transferase with a Hitachi 705 E analyzer (Hitachi, Tokyo, Japan) (based on Scandinavian recommendation[9]). The mean cell volume was determined with a Coulter S7 cell counter (Coulter Electronics, Hialeah, Florida).

Individual values of γ-glutamyl transferase and mean cell volume on any occasion were considered separately in different drinking groups. The relationship between degree of alcohol abuse and occurrence of fetal alcohol effects was interpreted as follows: true positive (TP) (marker high, abuse positive, fetal alcohol effects positive), false positive (FP) (marker high, abuse negative, fetal alcohol effects negative), true negative (TN) (marker normal, abuse negative, fetal alcohol effects negative), and false negative (FN) (marker normal, abuse positive, fetal alcohol effects positive). Sensitivity of the indices was TP/(TP + FN), specificity TN/(TN + FP), predictive value TP/(TP + FP), and relative risk factor, the ratio of TP/(TP + FP) to FN/(FN + TN). The significances of the differences were tested by binomial t test.

Results

The γ-glutamyl transferase (range 3 to 65 U/L) and mean cell volume (range 85 to 98 fl) in the control women did not change with advancing gestation.

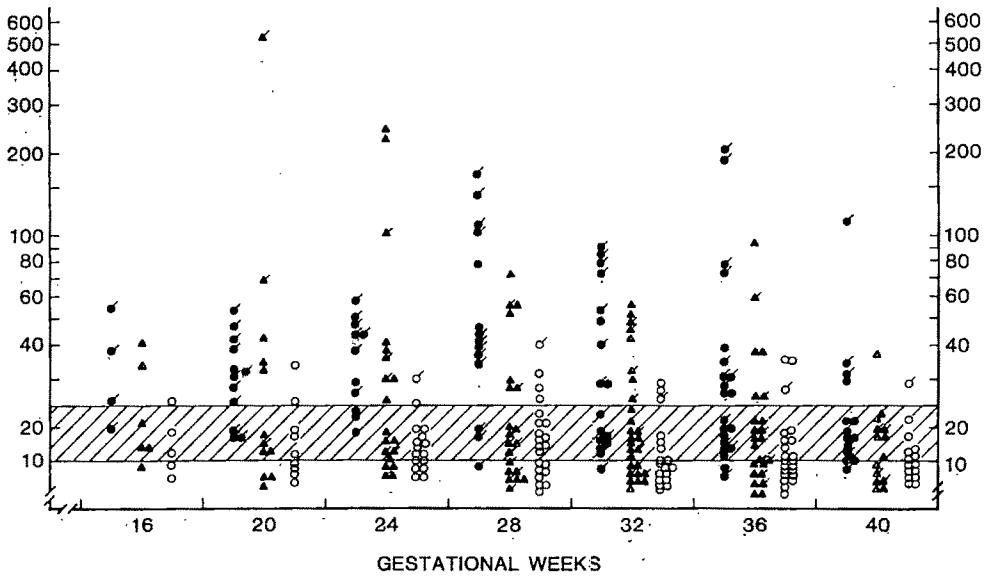


Fig. 1. Individual γ-glutamyl transferase activities (units per liter) in pregnant female alcohol abusers. The *shaded area* represents the mean ± 2 SD in 28 abstinent healthy pregnant women who had follow-up throughout pregnancy. Alcoholic women (●); heavy drinkers (▲); moderate drinkers (○). Note the logarithmic scale for γ-glutamyl transferase. ◐, ◭, and ♂ indicate values in women who gave birth to infants with alcohol effects.

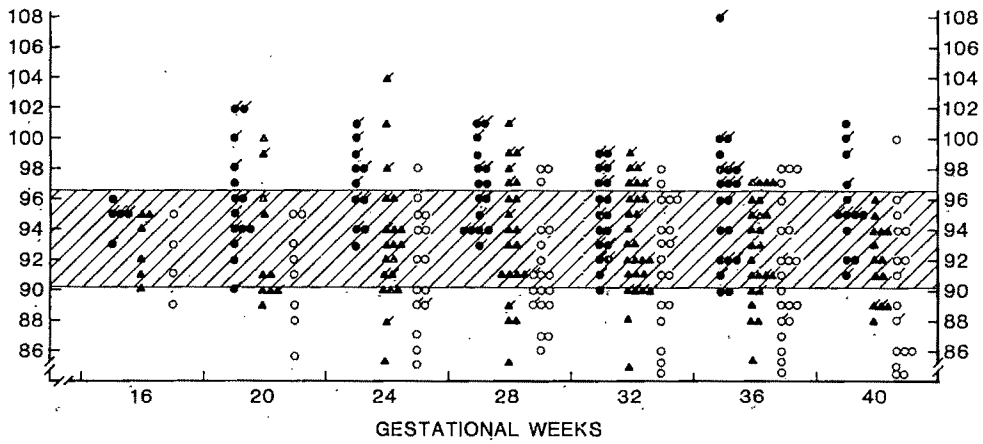


Fig. 2. Individual mean cell volumes (femtoliters) in pregnant women with alcohol abuse. See legend to Fig. 1 for symbols.

Table III. Efficacy of combined use of γ-glutamyl transferase and mean cell volume to reveal alcohol abuse during pregnancy

	Moderate drinkers (%)	*Heavy drinkers (%)*	*Alcoholic women (%)*	*All drinkers (%)*
Increased γ-glutamyl transferase, normal mean cell volume	15.9	13.3	30.1	19.1
Normal γ-glutamyl transferase, increased mean cell volume	12.1	6.7	17.2	11.6
Increased γ-glutamyl transferase and/or mean cell volume	27.1	38.3	76.3	45.6

Table IV. Sensitivity, specificity, predictive value, and relative risk of increased γ-glutamyl transferase and mean cell volume in predicting fetal alcohol effect

	γ-Glutamyl transferase	*Mean cell volume*	*Significance*
Sensitivity (%)	61.5	40.9	$p < 0.001$
Specificity (%)	78.5	87.9	$p < 0.01$
Predictive value (%)	59.3	65.3	NS
Relative risk	2.51	2.40	NS

NS = Not significant.

Therefore the mean + 2 SD was taken as the upper reference limit. These were 24.5 U/L for γ-glutamyl transferase and 96.6 fl for mean cell volume (Figs. 1 and 2). A total of 94.8% of the γ-glutamyl transferase values (128 of 135) and 95.6% of the mean cell volume values (132 of 138) in the control women were within the reference range.

Maternal alcohol abuse directly correlated with increased γ-glutamyl transferase and mean cell volume values (Figs. 1 and 2). Both markers revealed maternal alcohol abuse in relation to its severity, but the γ-glutamyl transferase was more sensitive (Table II). Increases in γ-glutamyl transferase and mean cell volume did not always coincide, and therefore the combined use of the markers resulted in the greatest efficacy in revealing alcohol abuse (Table III).

The rate of increased γ-glutamyl transferase and mean cell volume values decreased from 28 weeks of gestation onward (Fig. 3). A high γ-glutamyl transferase or large mean cell volume predicted the development of fetal alcohol effects (Figs. 1 and 2, Table IV). γ-Glutamyl transferase was more sensitive but less specific than mean cell volume, whereas predictive values and relative risks were fairly similar (Table III).

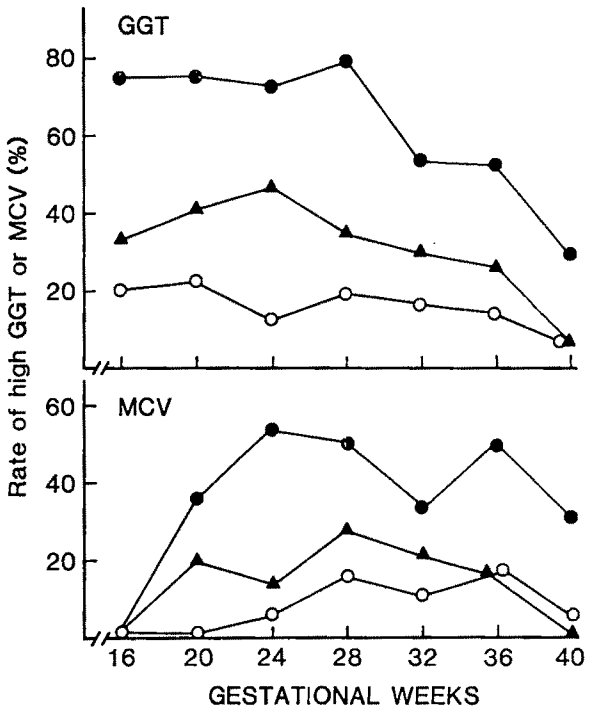


Fig. 3. The rate of high γ-glutamyl transferase *(GGT)* and mean cell volume *(MCV)* in pregnant alcoholics (●) and in women with heavy (▲) or moderate (○) alcohol use in relation to gestational age.

Comment

Approximately 40% of pregnant alcoholic women but only one of 600 to 700 unselected parturients give birth to infants with complete fetal alcohol syndrome; milder forms of fetal alcohol effects are at least twice as common.[8] An excessive amount of alcohol is the most common known teratogen today. The only way to prevent fetal alcohol effects is to identify problem drinkers before pregnancy or as soon as possible during pregnancy, when the fetus may not yet be damaged, and to counsel these women to refrain from alcohol use.

We assessed the usefulness of γ-glutamyl transferase and mean cell volume in revealing alcohol abuse in pregnant women who were known to have alcohol problems and who could have follow-up throughout pregnancy. Forty-two of the women (49%) had drunk so much before entering our project or drank so heavily after registration that their fetuses were damaged. From such cases we could also evaluate the efficacy of γ-glutamyl transferase and mean cell volume tests in predicting fetal alcohol effects.

Long-term excessive consumption of alcohol leads to increased serum γ-glutamyl transferase, either by direct damage to the liver cell or by the induction of microsomal enzymes in the absence of overt damage; it leads to increased mean cell volume by the direct action of ethanol on the developing erythroblasts.[1-6] In contrast to identification of blood ethanol level, these changes can remain detectable for weeks. Alcohol abuse often remains unrecognized during pregnancy because women drinkers seem to be able to refrain from drinking for a couple of days before follow-up visits at their prenatal centers. This was also seen in the present study, since only 7% of the blood samples contained detectable levels of ethanol (Halmesmäki E, unpublished data).

The γ-glutamyl transferase level is high in two thirds of pregnant alcoholic women and one third of pregnant heavy drinkers. This fits well with the data on nonpregnant alcoholic women of whom 50% to 80% have

increased γ-glutamyl transferase levels.[3, 5, 6] One study group has assessed the value of γ-glutamyl transferase as a screening method for alcohol consumption during the first trimester of pregnancy.[10] In this study 13% of 669 women had consumed more than 30 gm of ethanol per day, and two produced infants with fetal alcohol effects.[10] Only 25% of these women had an increased γ-glutamyl transferase value.[10] It is likely that the average alcohol consumption of our patients was higher than that in the previous study,[10] since much higher rates of fetal alcohol effects and increased serum levels of γ-glutamyl transferase were found in our study.

The mean cell volume test is a less sensitive marker than γ-glutamyl transferase for detection of alcohol abuse during pregnancy, as it also is in nonpregnant subjects.[3, 5, 6] This difference is at least partly explained by the long life span of erythrocytes. Alcohol-induced biochemical change that causes macrocytosis is fully reflected in the mean cell volume value only after 120 days. For this reason mean cell volume may reflect better the long-term alcohol effects, whereas γ-glutamyl transferase responds more rapidly. An increased mean cell volume also was found more often with advancing gestation (Fig. 3). This may suggest that in pregnancies complicated with alcohol abuse, the sensitivity of erythroblasts to ethanol increases. Folic acid deficiency and/or smoking may be factors in this regard because they both may lead to increased mean cell volume.

No prenatal diagnostic methods exist for fetal alcohol effects. An elevated γ-glutamyl transferase level predicts fetal alcohol effects relatively well, whereas the sensitivity of mean cell volume is low but the specificity high. In predicting fetal alcohol effects the sensitivity and specificity of these rather simple laboratory methods compare favorably with those of low levels of α-fetoprotein and β_1-glycoprotein in maternal serum.[11] Although prenatal measurement of γ-glutamyl transferase and mean cell volume can be used to evaluate the risk of fetal alcohol effects, we do not yet have any effective preventive treatments for subjects who cannot stop drinking.

The responses of γ-glutamyl transferase and mean cell volume to alcohol were not uniform, and mean cell volume was increased in 6.7% to 17.2% of the samples with normal γ-glutamyl transferase levels. A combined use of γ-glutamyl transferase and mean cell volume thus revealed the maternal alcohol abuse better than did either test alone. Furthermore, although the majority of patients (65%) reported a reduced alcohol use during follow-up visits (Halmesmäki E, unpublished data), γ-glutamyl transferase values remained high to 32 weeks of pregnancy, and high mean cell volume values became more frequent. This may imply that contrary to their own reports, the patients failed to reduce their alcohol use (and/or smoking) until the last 6 to 8 weeks of pregnancy. γ-Glutamyl transferase and mean cell volume measurements may therefore be used as objective indices of the efficacy of alcohol counseling in pregnant problem drinkers.

In conclusion, increased γ-glutamyl transferase and/or mean cell volume reveal maternal alcohol abuse during pregnancy so reliably that these markers should be determined routinely, at least in women with recognized or suspected alcohol problems.

REFERENCES

1. Wu A, Chanarin I, Levi AJ. Macrocytosis of chronic alcoholism. Lancet 1974;1:824.
2. Chick J, Kreitman N, Plant M. Mean cell volume and gammaglutamyltranspeptidase as markers of drinking in working men. Lancet 1981;1:1249.
3. Holt S, Skinner HA, Israel Y. Early identification of alcohol abuse: 2. Clinical and laboratory indicators. Can Med Assoc 1981;15:1279.
4. Papoz L, Warnet JM, Pequignot G, Eschwege E, Claude JR, Schwartz D. Alcohol consumption in a healthy population. JAMA 1981;245:1748.
5. Prevention of hazardous drinking: the value of laboratory tests [Editorial]. Br Med J 1985;240:1849.
6. Skinner HA, Holt S, Shew WJ, Israel Y. Clinical versus laboratory detection of alcohol abuse: the alcohol clinical index. Br Med J 1986;292:1703.
7. Jones KL, Smith DW. Recognition of the fetal alcohol syndrome in early infancy. Lancet 1973;2:999.
8. Streissguth AP, Landesman-Dwyer S, Martin JC, Smith DW. Teratogenic effects of alcohol in humans and laboratory animals. Science 1980;209:353.
9. Scandinavian Enzyme Committee. Recommended method for the determination of γ-glutamyltransferase in blood. Scand J Lab Invest 1976;36:119.
10. Larsson G, Ottenblad C, Hagenfeldt L, Larsson A, Forsgren M. Evaluation of serum γ-glutamyl transferase as a screening method for excessive alcohol consumption during pregnancy. AM J OBSTET GYNECOL 1983;147:654.
11. Halmesmäki E, Autti I, Granström M-L, Heikinheimo M, Raivio K, Ylikorkala O. α-Fetoprotein, human placental lactogen, and pregnancy-specific β_1-glycoprotein in pregnant women who drink: relation to fetal alcohol syndrome. AM J OBSTET GYNECOL 1986;155:598.

Progesterone synthesis by human amnion, chorion, and decidua at term

B. F. Mitchell, M.D., J. R. G. Challis, Ph.D., D.Sc., and L. Lukash, B.Sc.
London, Ontario, Canada

We investigated the ability of human fetal membranes to produce progesterone from a variety of substrates. Chorion is more active than decidua, and amnion produces little progesterone. Cholesterol or low-density lipoprotein cholesterol was not used as a substrate. Chorion used pregnenolone, pregnenolone sulfate, and 20α-dihydroprogesterone for progesterone synthesis. Decidua also used these three substrates but produced significantly less progesterone than chorion. Amnion used only 20α-dihydroprogesterone as a substrate. Exogenous human chorionic gonadotropin or gonadotropin-releasing hormone or its analogues did not influence progesterone production by any of the tissues. There were several significant changes in substrate usage for progesterone synthesis by the tissues around the time of the onset of labor. Tissue concentrations of progesterone are approximately 4 ng/mg protein, and it appears that local production rates could completely account for this. These data are compatible with the hypothesis that local regulatory mechanisms may determine progesterone concentrations in fetal membrane tissues and that important changes may occur around the time of the onset of parturition. (AM J OBSTET GYNECOL 1987;157:349-53.)

Key words: Progesterone, amnion, chorion, decidua

Several studies in recent years have demonstrated the ability of human fetal membranes to synthesize and metabolize progesterone.[1-5] We have hypothesized that local paracrine events may regulate tissue progesterone concentrations within the fetal membranes, and these in turn may modify myometrial contractility. Investigations in our laboratory have shown that pregnenolone can be used as a substrate for progesterone synthesis and that chorion is the most active tissue in this regard.[1,2] We also demonstrated that other steroid hormones can modify this reaction.[1,3] The studies presented here extend our observations into progesterone synthesis by human amnion, chorion, and decidua.

It is well established that human chorionic gonadotropin (hCG) stimulates progesterone production in luteal cells. Recent studies[6,7] have demonstrated an effect of gonadotropin-releasing hormones (GnRH) on placental progesterone production. Thus we have performed studies to investigate the potential regulation of pregnenolone conversion to progesterone by hCG and GnRH or its analogues.

From the Research Institute, St. Joseph's Hospital, Department of Obstetrics and Gynaecology, University of Western Ontario.
Presented in part at the Thirty-third Annual Meeting of the Society for Gynecologic Investigation, Toronto, Ontario, Canada, March 19-22, 1986.
Received for publication August 28, 1986; revised February 9, 1987; accepted March 5, 1987.
Reprint requests: B. F. Mitchell, M.D., Department of Obstetrics and Gynecology, University of Alberta, Edmonton, Alberta, Canada T6G 2R7.

Pregnenolone may be derived from two potential sources. It is well established that many tissues that secrete steroids use cholesterol associated with low-density lipoprotein as a source of substrate. Alternatively, pregnenolone may be produced from the hydrolysis of the high concentrations of pregnenolone sulfate, which are present in late human pregnancy.[8] In placental tissue progesterone also may be synthesized by conversion of one of its own metabolites, 20α-dihydroprogesterone.[9] We have investigated the ability of human fetal membrane tissues to use these various substrates for synthesis of progesterone.

If we are to hypothesize that local progesterone production within the fetal membranes may be an important regulator of local tissue progesterone concentrations, we must gain information concerning the amount of progesterone necessary to suffice local requirements. Thus we have measured endogenous progesterone concentrations within the amnion, chorion, and decidua at term.

Material and methods

Fetal membrane tissues were obtained immediately after delivery from women with uncomplicated pregnancies who gave birth at 38 to 42 weeks of gestation. Delivery occurred either after spontaneous onset of labor and vaginal delivery or by elective repeat cesarean section before the onset of labor. No oxytocics, narcotics, anesthetic agents, or antibiotics were used before delivery. Continuous epidural analgesia was provided for cesarean sections and most vaginal deliveries.

Table I. Effect of hCG and GnRH on progesterone synthesis from pregnenolone by dispersed cells

Substrate	*Chorion (n = 8)*	*Decidua (n = 4)*
Control	1.0 ± 0.5	0.2 ± 0.1
Pregnenolone (1 μg)	27.3 ± 4.2	6.6 ± 2.1
Pregnenolone + hCG (10 IU)	31.2 ± 7.0	5.3 ± 1.8
Pregnenolone + GnRH (10 ng)	24.5 ± 5.1	4.2 ± 1.3

Values are mean ± SEM (nanograms of progesterone produced per 10^5 cells per 4-hour incubation period).

Table II. Use of pregnenolone and cholesterol for progesterone synthesis by tissue explants (N = 6 patients)

Substrate	*Chorion*	*Decidua*
Control	31.5 ± 4.8	17.5 ± 3.2
Pregnenolone (1 μg)	1057 ± 213	673 ± 150
Cholesterol (μg)	30.7 ± 5.9	21.9 ± 5.4
Low-density lipoprotein cholesterol (5 μg)	27.6 ± 6.9	18.6 ± 4.7
Low-density lipoprotein cholesterol (50 μg)	36.6 ± 7.0	20.5 ± 3.8

Values are mean ± SEM (nanograms of progesterone produced per milligram of tissue protein per 24-hour incubation period).

The tissues were rinsed immediately in cold saline solution. The amnion was bluntly dissected from the choriodecidua and the decidua gently separated from the chorion. The tissues were either (1) minced and dispersed with 0.05% collagenase, as described previously,[1] or (2) cut into explants and incubated in minimal essential medium.[2] In both systems, two to four replicates were performed for each incubation. The dispersed cell incubations were conducted for 4 hours in room air at 37° C on a metabolic shaker. Each tube contained 75,000 to 200,000 cells (excluding red blood cells), and results were expressed as the amount of progesterone produced per 10^5 cells/4 hours. Cells were counted with a hemocytometer, and viability was assessed by trypan blue exclusion, which was always >90%. The tissue explants were incubated for 4 or 24 hours in 95% oxygen and 5% carbon dioxide at 37° C. Results were expressed as progesterone produced per milligram of tissue protein per 4 or 24 hours. Protein was estimated with the method of Lowry et al.[10]

To measure endogenous progesterone concentrations, tissue samples were obtained from sites near (within 2 cm) the edge of the placental disc, at the edge of the membranes most distant from the placenta, or an area midway between these two. The tissues were homogenized with a Brinkmann Polytron (Brinkmann Instruments, Inc., Westbury, New York). The crude homogenates were extracted twice with 5 volumes of diethyl ether. Extraction recovery was calculated with parallel tubes with the addition of radiolabelled progesterone. Extraction efficiency was >90%. The ether was evaporated and endogenous progesterone measured by radioimmunoassay.

All steroids were obtained from Steraloids Inc., Wilton, New Hampshire. The pregnenolone and 20α-dihydroprogesterone were recrystallized before use. The pregnenolone sulfate was extracted with ether before use to remove any spontaneously hydrolyzed product. The low-density lipoprotein cholesterol was prepared from human plasma by ultracentrifugation and was generously supplied by Dr. Murray Huff of the Department of Internal Medicine, University Hospital, London, Ontario, Canada. The GnRH and analogues were the generous gifts of Drs. E. R. Plunkett, Kalyan Sundaram and C. W. Bardin of the Population Council, New York, New York. The analogues tested included [(imBzl)-D-His^6,Pro^9-NEt]-GnRH; [D-Trp^6,Pro^9-NEt]-GnRH; [D-Ser-TBU)^6-NEt]-GnRH; [D-Trp^6]-GnRH; [AcD-Pro^1,pFD-Phe^2, D-$Trp^{3,6}$]-GnRH; [4CH_3-D-Phe^6,Pro^9-NEt]-GnRH; [4NO_2-D-Phe^6,Pro^9-Net]-GnRH, as well as native GnRH. The hCG was obtained commercially (A.P.L., Ayerst, Montreal, Quebec, Canada). Radiolabelled progesterone was obtained from New England Nuclear (North Billerica, Massachusetts).

The radioimmunoassay for progesterone has been validated previously for this system.[2] Assay blanks were subtracted where appropriate to correct for cross-reactivity of the substrates. Two to four replicates were performed for each incubation, and a single aliquot of each was assayed. Separate incubations were used to estimate recovery, and all data were corrected for recovery. Statistical comparisons were performed with a one-way analysis of variance.

Results

Dispersed cells from the chorion or decidua secreted progesterone, and this was increased markedly by the addition of pregnenolone to the incubations (Table I). As shown previously,[1,2] chorion was significantly more active in this regard than decidua. Amnion (data not shown) was almost devoid of such activity. The addition of hCG or GnRH had no significant effect on the progesterone production. In further experiments (data not shown), we determined that GnRH and seven analogues (see Material and methods) in concentrations of 10, 10^3, and 10^5 pg/ml had no effect on the conversion of pregnenolone to progesterone. In 24-hour incubations of tissue explants of chorion and decidua, the basal secretion of progesterone again was greatly increased by the addition of pregnenolone (Table II). However, we were unable to detect any use of choles-

Table III. Use of pregnenolone, pregnenolone sulfate, and 20α-dihydro-progesterone for progesterone synthesis by tissue explants (N = 10 patients in each group)

	Control	*Pregnenolone (1 μg)*	*Pregnenolone sulfate (1 μg)*	*20α-dihydro-progesterone (1 μg)*
Amnion				
Spontaneous labor	1.7 ± 0.2	1.4 ± 0.3	0.7 ± 0.1	13.6 ± 1.9*
Cesarean section	1.7 ± 0.7	1.0 ± 0.2	0.9 ± 0.2	20.7 ± 2.4*
Total group	1.7 ± 0.4	1.2 ± 0.2	0.8 ± 0.1	17.0 ± 1.6
Chorion				
Spontaneous labor	7.8 ± 2.2	98.3 ± 15.8	46.0 ± 6.9	31.9 ± 5.1
Cesarean section	7.4 ± 2.2	96.4 ± 10.8	43.2 ± 6.0	46.2 ± 8.7
Total group	7.6 ± 1.5	97.4 ± 9.6	44.7 ± 4.6	39.1 ± 5.1
Decidua				
Spontaneous labor	2.0 ± 0.5	11.2 ± 2.4*	6.0 ± 0.9*	4.4 ± 0.6
Cesarean section	2.1 ± 0.3	26.8 ± 6.3*	13.1 ± 2.5*	5.5 ± 0.8
Total group	2.1 ± 0.3	18.6 ± 3.4	9.4 ± 1.4	5.0 ± 0.5

Values are mean ± SEM (nanograms of progesterone produced per milligram of tissue protein per 4-hour incubation period).
*Significant ($p < 0.05$) difference between spontaneous labor and cesarean section groups by analysis of variance.

Table IV. Endogenous tissue concentrations of progesterone in human fetal membranes (N = 24 patients)

	Amnion	*Chorion*	*Decidua*	*Full thickness*
Spontaneous labor	3.1 ± 0.3	5.9 ± 1.0	4.6 ± 0.6	4.2 ± 0.2
Cesarean section	2.8 ± 0.5	4.6 ± 0.4	4.4 ± 0.7	4.3 ± 0.3
Total group	3.0 ± 0.3	5.3 ± 0.6	4.5 ± 0.4	4.3 ± 0.2

Values are mean ± SEM (nanograms of progesterone per milligram of tissue protein).

terol or low-density lipoprotein cholesterol for progesterone synthesis by either tissue. In these experiments we did not detect a difference in basal progesterone secretion or conversion of pregnenolone to progesterone between the tissues obtained from the patients who had spontaneous onset of labor compared with those from patients delivered by elective cesarean section before labor.

Use of other steroids for progesterone synthesis is shown in Table III. Tissue explants of amnion show very little basal secretion of progesterone in 24-hour incubations and demonstrate no ability to use pregnenolone or pregnenolone sulfate as a substrate. However, 20α-dihydroprogesterone is readily converted to progesterone, and tissues obtained from women who have a cesarean section before labor perform this activity better than those obtained from patients after spontaneous onset of labor and vaginal delivery.

Chorion explants used pregnenolone, its sulfate, and 20α-dihydroprogesterone for progesterone synthesis. The increased ability of tissues from cesarean section to use 20α-dihydroprogesterone approached statistical significance ($p = 0.08$). Decidua also was able to convert all three substrates to progesterone but to a much lesser extent than chorion. However, when provided with pregnenolone or its sulfate as substrate, the decidual explants obtained before labor secreted more progesterone than those obtained after spontaneous labor and vaginal delivery.

The data of Table III clearly established the relative ability of the three tissues to secrete progesterone. Chorion is greater than decidua, which is greater than amnion, except in the presence of 20α-dihydroprogesterone when amnion secretes more progesterone than decidua.

The endogenous tissue concentrations of progesterone are approximately 4 ng/mg of tissue protein (Table IV). In the 10 patients in either subgroup or in the total group, the concentration of progesterone was not different between chorion and decidua, but both were greater than amnion ($p < 0.05$). There was no significant change in tissue concentrations of progesterone with increasing distance from the placenta (Table V).

Comment

We have demonstrated that all three layers of human fetal membranes have the ability to secrete progesterone. Chorion is the most active layer in this regard but appears to show no significant change associated with the timing of the onset of labor. Decidua, though less active than chorion, does show a significant decline in the ability to produce progesterone associated with the onset of labor. The increased ability of decidua to use pregnenolone for progesterone production was not

Table V. Endogenous tissue concentrations of progesterone in full-thickness pieces of human fetal membranes in relation to distance from the placenta (N = 19 patients)

	Tissue progesterone concentration (ng/mg protein)	*Concentration found near the placental edge (%)*
Near placental edge	4.4 ± 0.4	
Middle	3.7 ± 0.4	88.1 ± 7.1
Distant from placenta	4.2 ± 0.5	101.6 ± 15.6

Values are mean ± SEM.

noted in the early experiments but only became apparent when larger patient groups were studied. Amnion tissue apparently possesses very little ability to produce progesterone except when provided with 20α-dihydroprogesterone as substrate. In this instance, there again appears to be a significant decline in progesterone secretion associated with the onset of labor.

The present experiments suggest that neither cholesterol nor low-density lipoprotein cholesterol are significant substrates for progesterone production by human fetal membranes. Even if longer incubations or long-term monolayer cultures of isolated cells were able to demonstrate low-density lipoprotein cholesterol usage, it would be unlikely to be of major physiologic importance in view of the apparent ability of the tissues to readily use other substrates that are available in abundant supply. Although chorion production of progesterone was greater with pregnenolone, in view of the much greater concentrations of pregnenolone sulfate available in late pregnancy, it may be that the sulfurylated form of the substrate is the most important physiologic progesterone precursor. Other studies in our laboratory[11] have demonstrated that the fetal membranes contain an active sulfohydrolase enzyme that possesses a lower Michaelis constant for pregnenolone sulfate than for either estrone sulfate or dehydroepiandrosterone sulfate. Currently it is unclear whether all sulfurylated steroids share the same sulfohydrolase enzyme or whether there are separate substrate-specific enzyme subtypes. The regulation of sulfohydrolase activity for pregnenolone sulfate may be an important determinant of ultimate progesterone secretion by these tissues.

Our present experiments were unable to detect any effect of hCG or GnRH on progesterone secretion. In this respect, the chorion trophoblast appears to behave in a different fashion than placental trophoblast, where progesterone production is influenced by GnRH.[6, 7] There remains some confusion as to whether progesterone secretion is increased[6] or decreased[7] by exogenous GnRH in cultures of placental trophoblast.

We have noted[1, 3] previously that androgens may inhibit the conversion of pregnenolone to progesterone. Estrone and estradiol also appear to have an inhibitory effect on progesterone production in dispersed cells,[1] but this was not observed with the tissue explant system.[3] It has been demonstrated that 5α-reduced metabolites such as 5α-dihydrotestosterone and 5α-pregnanediol, when coincubated with pregnenolone, lead to an increase in progesterone secretion. This observation with 5α-dihydrotestosterone suggests that the effect is caused by inhibition of the 5α-reduction metabolic pathway rather than an oxidation of the 5α-reduced metabolite back to progesterone. The potential importance of 20α-dihydroprogesterone as a precursor to progesterone is of interest. It has been demonstrated isotopically that this is caused by a conversion of the reduced metabolite back to progesterone rather than an inhibition of further metabolism of endogenously produced progesterone.[12] It has become apparent that the enzyme 20α-hydroxysteroid dehydrogenase, which catalyzes this interconversion, is the same active site on the same molecule as the enzyme 17-hydroxysteroid dehydrogenase that catalyzes the interconversion of estrone and estradiol.[13, 14] Thus the enzyme that inactivates progesterone by reduction at the 20 position is the same enzyme that increases the biologic activity of estrone by converting it to estradiol. A change in the velocity of this enzyme may have a significant effect on the biologic estrogen-progesterone activity within local tissues. Ongoing experiments in our laboratory involve the investigation of the kinetics of the 17,20-hydroxysteroid dehydrogenase enzyme.

When comparing the ability of the fetal membrane tissues to produce progesterone with the endogenous progesterone concentrations, it appears quite feasible that the local tissues are capable of synthesizing complete local requirements. The lack of demonstration of a gradient of progesterone with increasing distance from the placental edge suggests that simple diffusion of progesterone from the placenta does not account for local tissue concentrations. These findings are compatible with our hypothesis that regulation of local progesterone production may be the principle regulator of local progesterone tissue concentrations.

Although we were unable to demonstrate a change in tissue progesterone concentrations associated with the timing of the onset of labor, this does not preclude a significant change in progesterone concentrations at some important intracellular sites. Several lines of evidence indicate that progesterone may influence prostaglandin generation, and this in turn may determine myometrial contractility.[15] Thus it is possible that the provision of substrate and the activity of enzymes, particularly steroid sulfohydrolase and 17,20-hydroxysteroid dehydrogenase may regulate local tis-

sue progesterone concentrations, which in turn may influence the timing of the onset of parturition.

REFERENCES

1. Mitchell B, Cruickshank B, McLean D, Challis J. Local modulation of progesterone production in human fetal membranes. J Clin Endocrinol Metab 1982;55:1237.
2. Mitchell B, Powell W. Progesterone production by human fetal membranes: an in vitro incubation system for studying hormone production and metabolism. AM J OBSTET GYNECOL 1984;148:303.
3. Powell W, Mitchell BF, Challis JRG. Effects of steroids on progesterone output by explants of human chorion. Gynecol Obstet Invest 1986;22:64.
4. Milewich L, Gant NF, Schwarz BE, Chen GT, MacDonald PS. Initiation of human parturition. VIII. Metabolism of progesterone by fetal membranes of early and late human gestation. Obstet Gynecol 1977;50:45.
5. Gibb W, Lavoie JC, Roux FJ. 3β-hydroxysteroid dehydrogenase activity in human fetal membranes. Steroids 1980;32:365.
6. Siler-Khodr TM, Khodr GS, Valenzuela G, Rhode J. Gonadotropin-releasing hormone effects on placental hormones during gestation: II. Progesterone, estrone, estradiol and estriol. Biol Reprod 1986;34:255.
7. Branchaud CL, Goodyer CG, Lipowski LS. Progesterone and estrogen production by placental monolayer cultures: effect of dehydroepiandrosterone and luteinizing hormone-releasing hormone. J Clin Endocrinol Metab 1983;56:761.
8. de Peretti E, Mappus E. Pattern of plasma pregnenolone sulfate levels in humans from birth to adulthood. J Clin Endocrinol Metab 1983;57:550.
9. Palmer R, Blair JA, Eriksson G, Diczfalusy E. Metabolism of progesterone and 20α- and 20β-dihydroprogesterone by midterm placentas perfused in situ. Acta Endocrinol 1966;53:407.
10. Lowry OH, Rosebrough NJ, Farr LA, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265.
11. Mitchell BF, Chibbar R. Substrate utilization for progesterone synthesis in human fetal membranes [Abstract No. 113]. In: Proceedings of the thirty-third annual meeting of the Society for Gynecologic Investigation. Toronto, Ontario, Canada, March 19-22, 1986.
12. Powell WA, Challis JRG. Influence of 20α-dihydroprogesterone on progesterone output by human chorion explants. Gynecol Obstet Invest 1986;22:73.
13. Strickler RC, Covey DF, Tobias B. Study of 3α,20β-hydroxy-steroid dehydrogenase with an enzyme-generated affinity alkylator: dual enzyme activity at a single active site. Biochemistry 1980;19:4950-4.
14. Strickler RC, Tobias B. Estradiol 17β-dehydrogenase and 20α-hydroxysteroid dehydrogenase from human placental cytosol: one enzyme with two activities? Steroids 1980;36:243.
15. Thorburn GD, Challis JRG. Endocrine control of parturition. Physiol Rev 1979;59:863.

Antepartum predictors of fetal distress in postterm pregnancy

Clifford J. Bochner, M.D., Arnold L. Medearis, M.D., Jane Davis, M.D., Gary K. Oakes, M.D., Calvin J. Hobel, M.D., and Maclyn E. Wade, M.D.

Los Angeles, California

The purpose of this study was to determine the efficacy of combining nonstress testing with ultrasound assessment of amniotic fluid volume for the antenatal evaluation of the postterm fetus. Postterm patients (884) were managed with amniotic fluid assessments and nonstress tests (including evaluation for variable and late decelerations) twice a week. There were no perinatal deaths or major neonatal morbidity. However, the antenatal testing sensitivity, specificity, negative, or positive predictive values were not improved by combining the two tests. Individually, amniotic fluid assessment was just as accurate a predictor of fetal well-being and was a significantly more sensitive test than the nonstress test. In addition, antenatal predictors of fetal distress and intrapartum signs of fetal distress were almost exclusively those reflective of umbilical cord compromise. These findings stress the importance of antenatal screening for signs of umbilical cord compromise as an early indication of potential fetal compromise. Although the results also suggest that amniotic fluid assessment is superior to the nonstress test, they do not conclusively support the use of amniotic fluid assessment as the sole parameter for postterm antenatal surveillance. (AM J OBSTET GYNECOL 1987;157:353-8.)

Key words: Antepartum testing, nonstress test, amniotic fluid, variable decelerations, postterm pregnancy, fetal distress

From the Department of Obstetrics and Gynecology, Cedars-Sinai Medical Center.
Received for publication September 22, 1986, revised March 17, 1987; accepted April 27, 1987.
Reprint requests: Clifford J. Bochner, M.D., Department of Obstetrics and Gynecology, Cedars-Sinai Medical Center, 8700 Beverly Blvd., Los Angeles, CA 90048.

Antepartum fetal surveillance is used in postterm pregnancies in an attempt to avoid unnecessary intervention and to predict fetal compromise before permanent damage occurs.[1-4] Nonstress testing (NST) and contraction stress testing (CST) were designed to detect

Table I. Study population delivery and perinatal outcome separated according to the final antepartum testing results

Outcome	*Reactive NST and normal AF*			*Abnormal NST* and/or decreased AF*		*Total population*	
	N	%	*p Value*	*N*	%	*N*	%
Total patients	715			169		884	
C/S for fetal distress	6	0.8	0.0001	27	16.0	33	3.7
C/S for other indications	141	19.7	NS	27	16.0	168	19.1
1 min Apgar score <7	31	4.3	NS	16	9.5	47	5.3
5 min Apgar score <7	6	0.8	NS	2	1.2	8	0.9
Early labor heavy meconium	49	6.9	NS	18	10.7	67	7.6
Birth weight <10th percentile	44	6.2	NS	9	5.3	53	6.0
Major perinatal morbidity†	0	0.0		0	0.0	0	0.0
Mortality	0	0.0		0	0.0	0	0.0

AF = Amniotic fluid; C/S = culture and sensitivity; NS = not significant.

*Nonreactive and/or decelerations.

†Seizures, severe meconium aspiration, apnea, prolonged hospital stay.

antepartum signs suggestive of uteroplacental insufficiency, including the absence of fetal heart rate (FHR) reactivity and/or the presence of late decelerations.[1, 3] However, the use of these tests in postterm pregnancies has been criticized because of their documented high percentage of false positive results and instances of falsely reassuring results.[5, 6]

In recent years clinical studies have noted that there are additional antepartum signs suggestive of fetal compromise in postterm pregnancies, including variable decelerations on FHR tracings and decreased amniotic fluid on ultrasound examination.[2, 6-8] These findings have been associated with a significantly increased incidence of poor perinatal outcome even when the traditional FHR signs of uteroplacental insufficiency were not present.

The purposes of this study were (1) to determine whether the combined use of NST and ultrasound amniotic fluid assessment improves the accuracy of postterm antepartum testing and (2) to develop insight into the importance of postterm assessment of antepartum parameters reflective of umbilical cord compromise relative to parameters reflective of uteroplacental insufficiency.

Material and methods

The study population consisted of 884 uncomplicated cases of postterm patients referred to the Cedars-Sinai Medical Center's antepartum testing unit between January 1, 1983 and May 1, 1986. Patients were referred from its clinics, private practices, and an affiliated health maintenance organization. These patients had all reached 41 to 42 completed weeks of gestation. Gestational age was determined by using combinations of early dating criteria, including an accurate last menstrual period, first trimester uterine examination, timing of initial auscultated fetal heart tones, and first- or second-trimester ultrasound examination. To be included in the study patients had to be delivered within 4 days of antepartum testing, be beyond 42 completed weeks of gestation at delivery, and had to have received a trial of labor.

Antenatal evaluation. A standard fetal monitor equipped with an ultrasound transducer and external tokodynamometer was used to record uterine contractions, FHR, and fetal movements. Amniotic fluid volume was evaluated with an ADR linear-array real-time ultrasound scanner (Model 4000, Advanced Technology Limited, Seattle, Washington) by placing a transducer perpendicular to the uterine wall and measuring the largest vertical pocket of amniotic fluid.

NST interpretations were classified by the criteria of Keegan and Paul.[9] The CSTs were performed and classified according to the standard protocol and criteria of Freeman et al.[1] The FHR strips were also reviewed for bradycardias (decrease of 40 bpm or rate <90 bpm for longer than 1 minute[10]) and significant variable decelerations (decrease of at least 15 bpm from baseline for longer than 15 seconds, excluding those after an acceleration[11]). The amniotic fluid volume was considered adequate if at least one pocket measured 3 cm vertically and was considered decreased if the largest vertical pocket was <3 cm.

At this time there is no consensus regarding the ultrasound definition of decreased amniotic fluid. Previous studies[12] have shown an increased incidence of fetal distress related to vertical pockets <1 cm[4] or 2 cm.[12] Therefore, as a conservative measure, a 3 cm vertical pocket was chosen to minimize false negative results.

Table II. Comparison of NST versus amniotic fluid assessment, accurate for determining intrapartum fetal distress

	Test reults		*Outcome*				*Predictive value*	
Test	*Normal*	*Abnormal*	*Normal*	*Fetal distress*	*Sensitivity*	*Specificity*	*Negative %*	*Positive %*
Nonstress test* (including decelerations)	744	—	732	12	63.6	86.8	98.4	18.9
	—	140	119	21				
p Value					NS	<0.01	NS	—
Amniotic fluid assessment*	809	—	798	11	66.7	94.7	98.6	28.9
	—	75	53	22				

*p Value <0.0001.

Table III. Predictive values of amniotic fluid assessment in conjunction with NST results

	Amniotic fluid		*Outcome*		*Predictive values*	
NST results	*Normal*	*Decreased*	*Normal*	*Fetal distress N (%)*	*Negative (%)*	*Positive (%)*
Reactive	715	—	709	6 (0.8)	99.2	20.7
	—	29	23	6 (20.7)‡		
Nonreactive	39	—	38	1 (2.5)*	94.7§	22.7§
	—	22	17	5 (22.7)‡		
Reactive variables	32	—	30	2 (6.3)†	93.8‖	37.5§
	—	16	10	6 (37.5)‡		
Nonreactive variables	15	—	13	2 (13.3)‡	86.7¶	62.5§
	—	8	3	5 (62.5)‡		
Late decelerations	8	—	8	0	100	NA

*NS
†$p < 0.05$
‡$p < 0.001$
} As compared with the incidence of fetal distress in patients with a reactive NST and adequate amniotic fluid.

§NS
‖$p < 0.05$
¶$p < 0.01$
} As compared with the predictive values of amniotic fluid in conjunction with a reactive NST.

The twice-weekly antepartum testing protocol included amniotic fluid assessment, NSTs, and, when necessary, CSTs. The NSTs were performed for up to 40 minutes, with abdominal stimulation if the test was not reactive after 20 minutes, followed by amniotic fluid assessment. A nonreactive NST was repeated 2 hours later if the amniotic fluid was determined to be adequate and the cervix was unfavorable for induction. A CST was done if the NST was again nonreactive. If the CST was negative, patients were retested in 3 to 4 days. Abnormal testing that led to labor induction included (1) decreased amniotic fluid, (2) bradycardias or repetitive variable or late decelerations during the NST or CST, (3) a nonreactive NST in a patient subsequently noted to have an inducible cervix. Patients with normal testing were seen twice a week until they went into labor or labor was induced at the discretion of their personal physician.

All patients were managed with continuous electronic FHR monitoring throughout labor. Fetal distress in labor was defined clinically as either (1) repetitive late decelerations, (2) repetitive moderate to severe variable decelerations with either a pH of <7.20 or decreased variability, or (3) prolonged bradycardia. Monitor tracings were classified in accordance with definitions described by Freeman and Garite.[13]

Data analysis. The sensitivity, specificity, negative, and positive predictive values for fetal distress were calculated for the NST and amniotic fluid volume assessment, individually and combined. Statistical analyses were done with χ^2, Fisher's exact test, or McNemar's test, as appropriate. A p value <0.05 was considered statistically significant.

Results

The intrapartum and neonatal outcomes for the study population are presented in Table I. The last NST and amniotic fluid assessment were normal in 715 (80.9%) patients and abnormal in 169 (19.1%). Of the 169 patients with abnormal testing results, 29 had decreased amniotic fluid, 39 had nonreactive NSTs, 32 had variable decelerations, eight had late decelerations,

Table IV. Predictive values of NST parameters in conjunction with amniotic fluid results

	NST			*Outcome*			*Predictive values*		
					Fetal distress			*Positive (%)*	
Amniotic fluid	*R*	*NR*	*Var Decel*	*Normal*	*N*	*%*	*Negative (%)*	*N*	*Var Decel*
Normal	715	—	—	709	6	0.8	99.2	2.6	8.5
	—	39	—	38	1	2.5*			
	—	—	47	43	4	8.5†			
Decreased	29	—	—	23	6	20.7‡	79.3	22.7§	45.8§
	—	22	—	17	5	22.7‡			
	—	—	24	13	11	45.8‡			

R = Reactive; NR = nonreactive; Var Decel = variable decelerations.

*NS
†$p < 0.05$
‡$p < 0.001$
} As compared with the incidence of fetal distress in patients with a reactive NST and adequate amniotic fluid.

§$p < 0.01$, As compared with the predictive values of the NST result in conjunction with normal amniotic fluid.

Table V. Comparison of the sensitivities of combinations of antepartum testing parameters to detect patients with fetal distress

	Patients with fetal distress	
	N	*%*
Total	33	100.0
Any abnormal testing parameters	27	82.0
Decreased AF, variable decelerations	26	78.8*
Decreased AF, NR NST	23	69.7*
Decreased AF	22	66.7*§
Abnormal NST (NR, decelerations)	21	63.6†‖
Variables	15	45.5‡
NR NST	13	39.4‡

AF = Amniotic fluid; NR = nonreactive.

*NS
†$p < 0.01$
‡$p < 0.001$
} As compared with the sensitivity with all abnormal test parameters.

§NS
‖$p < 0.01$
} Compared with decreased AF and variable decelerations.

and 61 had combinations of more than one abnormal parameter. There were no perinatal deaths or major neonatal morbidity, including seizures, severe meconium aspiration, or apnea. However, the group with abnormal final antepartum testing results did have a significantly higher incidence of cesarean section for fetal distress as compared with the group with normal testing ($p < 0.001$). Of the 33 patients who had cesarean sections for fetal distress, 32 had either moderate-to-severe variables or prolonged bradycardias, and one had combined repetitive variable and late decelerations. The NST (including evaluation for decelerations) is compared with amniotic fluid assessment in Table II. For both tests there was a highly significant difference in the incidence of fetal distress when the last test was abnormal as compared with normal ($p < 0.01$). Both tests had extremely accurate negative predictive values (>98%) and poor positive predictive values (<30%). Although amniotic fluid assessment had a significantly greater sensitivity than the NST ($p < 0.01$), the specificities and negative predictive values were not significantly different between the tests. It was not appropriate to statistically compare the positive predictive values because patients were not randomized to alternate management schemes.

The predictive values of amniotic fluid assessment in conjunction with a reactive or abnormal NST and the NST in conjunction with adequate or decreased amniotic fluid are shown in Tables III and IV.

The negative predictive value of a reactive NST combined with adequate amniotic fluid was not significantly greater than that of either test alone (Tables II to IV). The positive predictive values of decreased amniotic fluid, a nonreactive NST, variable decelerations, and late decelerations were 20.7%, 2.5%, 6.3%, and 0%, respectively, when each of the other parameters were normal (Table IV).

When amniotic fluid was adequate, its negative predictive value was not significantly different whether the NST was reactive or nonreactive (Table IV). However, its negative predictive value was significantly less when the NST contained variable decelerations. When amniotic fluid was decreased, its positive predictive value was not significantly different, whether the NST was reactive, nonreactive, or contained decelerations (Table IV). The negative predictive value of a reactive NST was significantly less when amniotic fluid was decreased, whereas the positive predictive value of an abnormal NST was significantly less when amniotic fluid was adequate (Table IV).

The testing sensitivities of the abnormal antepartum

testing parameters are listed in Table V. The abnormal NST parameters combined with decreased amniotic fluid helped to detect 27 (82%) of the patients with eventual fetal distress. This was a significantly greater percentage than was identified by the NST alone ($p < 0.01$) but was not a significantly greater percentage than was identified by amniotic fluid assessment alone. The combination of decreased amniotic fluid and/or variable decelerations helped to detect 26 of these 27 patients.

Comment

The overall incidence of fetal distress and neonatal morbidity in this study was minimal, even though there was a significantly increased incidence of fetal distress in the abnormal testing group. This suggests that intervention in the abnormal testing group was undertaken before significant fetal compromise. Although it is tempting to credit antepartum testing for these excellent results, the low incidence of perinatal compromise may instead be the result of the fact that this was a closely followed population, in which patients considered to be at high risk were delivered before 42 completed weeks of gestation.

However, the study does stress the importance of postterm antenatal screening for signs of umbilical cord compromise as an early indicator of potential fetal compromise, relative to signs of uteroplacental insufficiency. This is demonstrated by the fact that of all the patients with eventual intrapartum fetal distress who were correctly identified by antenatal testing, decreased amniotic fluid and/or variable decelerations detected all but one (Table V). In addition, decreased amniotic fluid and variable decelerations were associated with a significantly increased incidence of fetal distress, even when the NST was reactive (Table III). Conversely, nonreactive NSTs or late decelerations were the only abnormal parameters in just 28% of the abnormal tests, and they were not associated with an increased incidence of fetal distress as long as amniotic fluid was adequate.

The intrapartum FHR tracing signs of fetal distress were reflective of these findings. Of the 33 cesarean sections for fetal distress, 97% were done because of either prolonged bradycardias or moderate to severe variable decelerations. These findings further support and extend to the antepartum period the findings of Leveno et al.,[14] associating the pathophysiology of postterm fetal distress almost exclusively with signs reflective of umbilical cord compression. As discussed by Leveno et al.,[14] this does not exclude uteroplacental insufficiency as the primary cause of postterm fetal compromise. It may be that antepartum signs of umbilical cord compromise are just an earlier reflection of uteroplacental insufficiency than the absence of FHR reactivity or the appearance of late decelerations.

This study also suggests that antepartum testing accuracy is not improved by combining the NST with amniotic fluid assessment. Although the NST and amniotic fluid assessment were each highly accurate predictors of fetal well-being, their accuracy was not improved when the tests were combined (Tables II and III). In addition, neither adequate amniotic fluid nor a reactive NST provided adequate assurance of fetal well-being to avoid pregnancy intervention when the other test was abnormal. The only exception was that adequate amniotic fluid, in the presence of a nonreactive NST without variables, was as reassuring of fetal well-being as when the NST was reactive. Individually and combined, these tests were poor predictors of intrapartum fetal distress. However, the low incidence of fetal distress in this population makes it extremely difficult to design a test with a high positive predictive value without having a detrimental effect on the testing specificity and negative predictive values. Lastly, the sensitivity of the combined tests was not statistically improved over the use of amniotic fluid assessment alone.

Because morbidity was minimal and the results did not show any statistical improvement to testing accuracy from combining the two tests, we considered whether one of these tests could be used alone as the sole means of postterm surveillance. Individually, amniotic fluid assessment was an extremely accurate predictor of fetal well-being (98.6%), and it was a significantly more sensitive test than the NST. These findings, as well as the excellent results obtained by Crawley et al.,[8] who used only amniotic fluid assessment, and the fact that amniotic fluid assessment is a fast and inexpensive procedure suggest that it is a superior test to the NST for postterm fetal surveillance. However, the results of this study do not conclusively support the use of amniotic fluid assessment alone for postterm fetal surveillance. We cannot discount that a prospective randomized study may show that amniotic fluid assessment combined with either an NST, or FHR screening for variable decelerations has a greater testing accuracy than amniotic fluid alone. Perhaps the results of this study and the one by Crawley et al.[8] will permit such a prospective study.

REFERENCES

1. Freeman RK, Garite T, Modanlou H, et al. Postdate pregnancy: utilization of the contraction stress test for pregnancy surveillance. Am J Obstet Gynecol 1981;140:128.
2. Eden RD, Gergely RZ, Schifrin BS, et al. Comparison of antepartum testing for postdate pregnancy. Am J Obstet Gynecol 1982;144:683.
3. Rochard F, Schifrin BS, Sureau C. Non-stressed fetal heart monitoring in the antepartum period. Am J Obstet Gynecol 1976;126:699.
4. Manning FA, Platt LD, Sipos L. Antepartum fetal evaluation: development of a biophysical profile. Am J Obstet Gynecol 1980;136:787.

5. Evertson LR, Gauthier RJ, Collea JV. Fetal demise following a negative contraction stress test. Obstet Gynecol 1978;51:671.
6. Miyazaki FS, Miyazaki BA: False reactive non-stress test in posterm pregnancy. Am J Obstet Gynecol 1981;140: 269.
7. Phelan JP, Platt L, Yeh S, et al. Continuing role of the NST in the management of postdates pregnancy. Obstet Gynecol 1984;64:624.
8. Crawley P, O'Herlihy C, Boylan P. The value of ultrasound measurement of amniotic fluid volume in prolonged pregnancy. Br J Obstet Gynaecol 1984;91:444.
9. Keegan KA, Paul RH. Antepartum fetal heart rate testing: IV. The nonstress test as the primary approach. Am J Obstet Gynecol 1980;136:75.
10. Druzin ML, Gratacos J, Keegan KA, et al. Antepartum fetal heart rate testing: VII. The significance of fetal bradycardia. Am J Obstet Gynecol 1981;139:194.
11. Timor-Tritsch IE, Dierker LJ, Zador I, et al. Fetal movements associated with fetal heart rate accelerations and decelerations. Am J Obstet Gynecol 1978;131:276.
12. Chamberlain PE, Manning FA, Morrison I, et al. Ultrasound evaluation of amniotic fluid volume: the relationship of marginal and decreased amniotic fluid volumes to perinatal outcome. Am J Obstet Gynecol 1984;150:250.
13. Freeman RK, Garite TJ. Fetal heart rate monitoring. Baltimore: Williams & Wilkins, 1981:63.
14. Leveno KJ, Quirk JG, Cunningham FG, et al. Prolonged pregnancy: observations concerning the causes of fetal distress. Am J Obstet Gynecol 1984;150:465.

Ultrasound diagnosis of abruptio placentae with fetomaternal hemorrhage

Michael S. Cardwell, M.D.
Rockford, Illinois

Fetomaternal hemorrhage and abruptio placentae may occur concurrently. A prospective study was done to determine the incidence of fetomaternal hemorrhage in noncatastrophic cases of abruptio placentae consistent with sonographic findings. Significant fetomaternal hemorrhage was found in 75% of noncatastrophic cases. Implications of conservative management of abruptio placentae with fetomaternal hemorrhage are discussed. (Am J Obstet Gynecol 1987;157:358-9.)

Key words: Abruptio placentae, fetomaternal hemorrhage

Fetomaternal hemorrhage and abruptio placentae are two relatively common complications of pregnancy. Fetomaternal hemorrhage occurs in approximately 50% of all pregnancies and abruptio placentae has an incidence of 1/120 deliveries.[1] Fetomaternal hemorrhage can occur as a result of abruptio placentae as there is a breach in the integrity of the placental circulation.

In some instances abruptio placentae may occur without significant clinical signs and may be managed conservatively. These cases usually occur in early pregnancy and the diagnosis is confirmed by ultrasound examinations. To determine the relationship between ultrasound findings of abruptio placentae and fetomaternal hemorrhage in noncatastrophic cases a prospective study was done.

From the Rockford Memorial Hospital.
Received for publication January 21, 1987; accepted February 3, 1987.
Reprint requests: Michael S. Cardwell, M.D., Maternal-Fetal Medicine, Baptist Hospital, 2000 Church St., Nashville, TN 37236.

Material and methods

The study period was from July 1, 1985, through June 30, 1986. All patients with ultrasound examinations performed in the Department of Maternal-Fetal Medicine in which marked sonographic appearance of abruptio placentae was present in association with clinical signs and symptoms were chosen for the study. During this period 1225 consecutive ultrasound examinations with the use of a Toshiba SAL-50 real-time linear scanner with either a 4.0 or 5.0 MHz transducer were performed exclusively by M. S. C. for a variety of indications.

The ultrasound diagnosis of abruptio placentae was made if a retroplacental lucency suggestive of pooled blood or clot was apparent or if separation of the chorion-amnion from the uterine wall was present. Fetomaternal hemorrhage was evaluated with the Kleihauer-Betke stain. The results of the stain were reported as a ratio of fetal cells to adult cells. The quantity of the fetomaternal hemorrhage was determined by the formula derived by Mollison[2]:

$$\text{Fetomaternal hemorrhage (ml fetal red blood cells)} = 2400 \times \frac{\text{fetal cells}}{\text{adult cells}}$$

Results

Eight patients had both sonographic and clinical findings suggestive of abruptio placentae. The study group comprised these patients. Four patients presented with catastrophic indications for immediate delivery by cesarean section, for example, fetal distress, severe vaginal bleeding, or severe pain. No Kleihauer-Betke staining was performed in these patients because of the urgency for immediate delivery. The other four patients presented with clinical and sonographic evidence of abruptio placentae but the condition was not life-threatening to the mother or the fetus. Kleihauer-Betke stains were used to determine the presence of fetomaternal hemorrhage in the noncatastrophic group.

The two groups differ in several aspects. The catastrophic group had gestational ages ranging from 29 to 34 weeks, with a mean of 31.8 weeks. The noncatastrophic group's gestational age varied between 14 and 31 weeks with a mean of 21.8 weeks. The percentage of placental surface estimated by the attending obstetrician to have been separated in the catastrophic group varied from 20% to 50% of the total area. The mean separation was 35%. Only one patient in the noncatastrophic group had evidence of abruptio placentae at the time of delivery. The mean gestational age at delivery was 37.5 weeks for the noncatastrophic group and 31.8 weeks for the catastrophic group. Neither group experienced a neonatal death.

Three of four patients in the noncatastrophic group had evidence of fetomaternal hemorrhage. The transplacental hemorrhage ranged from no fetal blood cells to 72 ml of fetal blood cells detected with a mean of 31.5 ml of fetal red blood cells. One patient required 6 ampules of RhoGam to protect her from Rh isoimmunization.

Comment

In this prospective study abruptio placentae was diagnosed in eight of 1225 ultrasound examinations done for a variety of indications. This gives an incidence of 0.65%, which agrees with the cited occurrence of abruptio placentae of 0.49% to 1.29%.[1] Fetomaternal hemorrhage was verified in 75% of the noncatastrophic cases and was demonstrated to be significant with an average fetal red blood cell loss of 31.5 ml. Fetomaternal hemorrhage is known to occur in approximately 50% of all pregnancies but in less than 1% of the patients is the bleeding >5 ml.

The outcomes of the mothers and infants in this series were generally good; however, serious complications can occur with fetomaternal hemorrhage. Fetomaternal hemorrhage has been associated with antepartum intrauterine fetal death, neonatal anemia, anaphylatic shock of the mother, and sensitization to the Rh system and other blood groups. Hydrops fetalis and intrauterine fetal atrial fibrillation has also been reported in association with fetomaternal hemorrhage. If conservative management is elected in patients with noncatastrophic abruptio placentae, documentation and quantification of fetomaternal hemorrhage are imperative.

REFERENCES

1. Knab DR. Abruptio placenta: an assessment of the time and method of delivery. Obstet Gynecol 1978;52:625.
2. Mollison PL. Quantitation of transplacental hemorrhage. Br Med J 1972;3:31.

Acute atherosis in preeclampsia: Maternal determinants and fetal outcome in the presence of the lesion

T. Y. Khong, M.B., M.Sc., J. M. Pearce, and W. B. Robertson, M.D.
London, England

In 39 patients with preeclampsia a detailed analysis of some maternal factors, fetal outcome, and a histologic assessment of the presence or absence of acute atherosis in maternal intrauterine vessels has been done. There was no statistically significant relation between acute atherosis and parity, degree of proteinuria, severity and duration of hypertension, or antihypertensive therapy. These findings do not support a purely hemodynamic pathogenesis for the vasculopathy. Fetal outcome was only marginally worse in the preeclamptic patients who developed acute atherosis. (Am J Obstet Gynecol 1987;157: 360-3.)

Key words: Acute atherosis, preeclampsia, maternal factors, fetal outcome

A defective maternal vascular response to placentation has been demonstrated in pregnancies complicated by preeclampsia and by small for gestational age infants.[1-3] In addition, a vasculopathy called acute atherosis[4] is seen in intrauterine vessels in preeclampsia.[5] The arteries undergo fibrinoid necrosis accompanied by lipophage infiltration of the damaged vessel wall and a perivascular mononuclear cellular infiltrate.[6-9]

The significance of this lesion has been highlighted by a recent study that showed a lower birth weight in pregnancies when acute atherosis is found in vessels in the placental bed.[10] It also has been suggested[8] that these lesions offer the best explanation for the decidual necrosis and subsequent abruptio placentae and antepartum hemorrhage and for maternal vascular thrombosis leading to placental infarction.

The pathogenesis of acute atherosis is still unclear. Inappropriate immunologic fetal-maternal interactions have been postulated.[5, 8, 11-13] Other studies have suggested that preeclampsia associated with acute atherosis is more severe than that without atherosis[6] and that acute atherosis is seen with increasing frequency as the severity of the preeclampsia increases.[7] To evaluate the role of hemodynamic factors in the pathogenesis of acute atherosis and the relation of the lesion to maternal and fetal factors, we undertook a retrospective study of the maternal and fetal parameters in preeclamptic pregnancies.

From the Departments of Histopathology and Obstetrics, St. George's Hospital Medical School.
Received for publication December 16, 1986; revised March 19, 1987; accepted March 30, 1987.
Reprint requests: T. Y. Khong, M.B., Department of Histopathology, John Radcliffe Hospital, Oxford, OX3 9 DU, United Kingdom.



Fig. 1. Acute atherosis in a maternal vessel in the decidua parietalis showing fibrinoid necrosis, infiltration by lipophages, and a perivascular mononuclear cellular infiltrate. (Hematoxylin and eosin stain. ×200.)

Material and methods

The case notes on 39 consecutive preeclamptic pregnancies from which material had been submitted

Table I. Maternal factors and relation to acute atherosis in preeclampsia

	With acute atherosis (n = 18)	*Without atherosis (n = 21)*	*Significance*
Gravidity			
1	7	12	
2	6	6	
3	4	2	NS
>4	1	1	
Preexisting hypertension	3	2	NS
Mean arterial pressure (mm Hg) (mean ± 1 SD)	135.5 ± 13.5	132.6 ± 14.8	NS
Maximum systolic pressure (mm Hg)	177.5 ± 18.3	170.2 ± 22.5	NS
Maximum diastolic pressure (mm Hg)	115.3 ± 12.8	113.8 ± 11.9	NS
Maximum rise in systolic blood pressure (mm Hg)	58.3 ± 19.5	54.3 ± 22.9	NS
Maximum rise in diastolic blood pressure (mm Hg)	38.8 ± 12.6	41.7 ± 13.1	NS
Duration of rise in blood pressure (mm Hg)			
1-3 days	3*	6	
4-7 days	4	3	
8-14 days	3	3	NS
>14 days	5	9	
Proteinuria			
Trace	1	1	
1+	0	2	
2+	3	2	
3+	5	5	NS
4+	9	11	
Therapy required	16	15	NS
Gestation at delivery (wk) (mean ± 1 SD)	30 ± 2.6	33.6 ± 4.0	$p < 0.001$

NS = Not significant.
*Three not known since not booked before admission.

Table II. Fetal outcome and relation to acute atherosis in preeclampsia

Outcome	*With acute atherosis*	*Without atherosis*	*Significance*
Mean birth weight percentile (range)	10.8 (2-49)	18.7 (1-53)	NS
Fetal distress	11	10	NS
Intrauterine growth retardation	6	8	NS
Small for gestational age	10	7	NS
Stillborn	3	0	NS
Neonatal death	0	4	NS

NS = Not significant.

for histologic assessment were studied. Preeclampsia was defined as proteinuric hypertension of >140/90 mm Hg on two occasions at least 6 hours apart. Parity, gestational age at delivery, maximum blood pressure, degree of rise in blood pressure, degree of proteinuria, interval between the onset of proteinuric hypertension and delivery, and the use of antihypertensive therapy were studied. Gestational age was calculated in early pregnancy by ultrasound measurement of the biparietal diameter or by dates of the last menstrual period. Mean arterial pressure was calculated according to Page and Christianson.[14] Proteinuria was detected by the use of a multireagent dipstick in all cases, and the maximal dipstick reading was used. In addition, the birth weight percentile, evidence of fetal distress on cardiotocography or fetal blood sampling, intrauterine growth retardation, and fetal death were recorded. The birth weight chart of Milner and Richards[15] was used for births between 28 and 32 weeks of gestation and that of Altman and Coles[16] for gestations after 33 weeks. Three twin pregnancies and three deliveries before 28 weeks of gestation were not studied for the birth weight percentile. Intrauterine growth retardation was separated into true intrauterine growth retardation on the basis of sequential ultrasonographic studies and small for gestational age infants when the birth weight was below the tenth percentile, but sequential ultrasonographic studies were not available to confirm actual growth retardation.

Because placental bed biopsies were not always available,[17] it was decided that the delivered placentas were to be used as the study material. From each placenta

numerous blocks (nine to 12) of the basal plate and between two and four amniochorion rolls, prepared according to the method of Zeek and Assali,[6] were examined histologically. From this assessment the pregnancies were classified into those with and those without acute atherosis.

The maternal data of the two groups were analyzed for differences. When appropriate, the differences between mean values were compared with the two-tailed t test for unpaired data, and differences between proportions were compared with the χ^2 test. Tests were considered significant at the 5% level.

Results

Acute atherosis (Fig. 1) was detected in maternal vessels in 18 of the 39 patients (46%). Except for gestational age at delivery, when there was a statistical difference, analysis of the maternal parameters showed no significant differences between the two groups of patients (Table I). No statistical differences in the fetal outcome between the two groups could be demonstrated (Table II). There were three stillbirths in the acute atherotic group and four neonatal deaths in the nonacute atherotic group.

Comment

Because acute atherosis is focal, it may be expected that false negative findings of absence of acute atherosis would be encountered because of selection factors and the unavailability of placental bed biopsies from all cases. However, we believe that the large number of blocks taken from each placenta was sufficient to enable the majority of acute atherotic lesions to be detected if they were present. Our incidence of acute atherosis in preeclampsia (46%) is in accordance with the findings of other workers who used placental and placental bed material that ranged from 41.8% to 47.8%.[6, 12] Maqueo et al.[7] found the incidence to be 22%, 42%, and 65% in mild and severe preeclampsia and in eclampsia, respectively.

The mean gestational age at the time of delivery was almost 4 weeks earlier in the acute atherotic group; this may be a reflection of the somewhat poorer fetal outcome in this group. The incidence of fetal distress, small for gestational age infants, low birth weight percentiles, and stillbirth rate was higher in the acute atherotic group but that of intrauterine growth retardation was lower; however, these differences did not attain statistical significance. This finding contrasts with a recent study[10] that shows a lower mean birth weight in preeclamptic pregnancies with acute atherosis, but those results may be due to the failure of the authors to examine the maternal vessels attached to the amniochorion. Maqueo et al.[7] found an increased incidence of acute atherosis and a decreasing birth weight with severity of the disease, but the gestational ages in their cases decreased concomitantly. The stillbirth rates of their patients with mild and severe preeclampsia were not statistically different.

Although the number of cases studied may be small, analysis of the maternal factors did not support the concept that hypertension in itself causes acute atherosis or that the incidence of acute atherosis increased with the severity or duration of the condition.[6, 7] It may be that an inappropriate immune reaction is the major determinant of acute atherosis,[5, 8, 11-13] but proof for this proposal will require further detailed studies, including human leukocyte antigen typing of the mother, the father, and the fetus.

We wish to thank Dr. J. M. Bland of the Department of Clinical Epidemiology for his advice on the statistics.

REFERENCES

1. Brosens I, Robertson WB, Dixon HG. The role of the spiral arteries in the pathogenesis of pre-eclampsia. Obstet Gynecol Annu 1972;1:177-91.
2. Sheppard BL, Bonnar JL. The ultrastructure of the arterial supply of the human placenta in pregnancy complicated by fetal growth retardation. Br J Obstet Gynaecol 1976;83:948-59.
3. Khong TY, De Wolf F, Robertson WB, Brosens I. Inadequate maternal response to placentation in pregnancies complicated by preeclampsia and by small-for-gestational-age infants. Br J Obstet Gynaecol 1986;93:1049-59.
4. Sexton LI, Hertig AT, Reid DE, Kellogg FS, Patterson WS. Premature separation of the normally implanted placenta. Am J Obstet Gynecol 1950;59:13-24.
5. Robertson WB, Brosens I, Dixon HG. The pathological response of the vessels of the placental bed to hypertensive pregnancy. J Pathol Bacteriol 1967;93:581-92.
6. Zeek PM, Assali NS. Vascular changes in the decidua associated with eclamptogenic toxemia of pregnancy. Am J Clin Pathol 1950;20:1099-109.
7. Maqueo M, Azuela CJ, de la Vega MD. Placental pathology in eclampsia and preeclampsia. Obstet Gynecol 1964;24:350-6.
8. Robertson WB, Brosens I, Dixon G. Uteroplacental vascular pathology. Eur J Obstet Gynecol Reprod Biol 1975;5:47-65.
9. De Wolf F, Robertson WB, Brosens I. The ultrastructure of acute atherosis in hypertensive pregnancy. Am J Obstet Gynecol 1975;123:164-74.
10. McFadyen IR, Price AB, Geirsson RT. The relation of birth weight to histological appearances in vessels of the placental bed. Br J Obstet Gynaecol 1986;93:476-81.
11. Kitzmiller JL, Benirschke K. Immunofluorescent study of placental bed vessels in preeclampsia. Am J Obstet Gynecol 1973;115:248-51.
12. Hustin J, Foidart JM, Lambotte R. Maternal vascular lesions in pre-eclampsia and intrauterine growth retardation. Light microscopy and immunofluorescence. Placenta 1983;4:489-98.
13. Labarerre C, Althabe O. Chronic villitis of unknown etiology and maternal lesions in preeclamptic pregnancies. Eur J Obstet Gynecol Reprod Biol 1985;20:1-11.
14. Page EW, Christianson R. The impact of mean arterial pressure in the middle trimester upon the outcome of pregnancy. Am J Obstet Gynecol 1976;125:740-5.
15. Milner RDG, Richards B. An analysis of birth weight by

gestational age of infants born in England and Wales, 1967 to 1971. J Obstet Gynaecol Br Commonw 1980;81: 956-67.
16. Robertson WB, Khong TY, Brosens I, De Wolf F, Sheppard BL, Bonnar J. The placental bed biopsy. Review from three European centers. AM J OBSTET GYNECOL 1986;155:410-2.

Increased fetoplacental active renin production in pregnancy-induced hypertension

Harbinder S. Brar, M.D., Siri L. Kjos, M.D., William Dougherty, M.D., Yung-Shun Do, Ph.D., Helen B. Tam, and Willa A. Hsueh, M.D.*
Los Angeles, California

Since pregnancy-induced hypertension is associated with impaired uteroplacental blood flow, we studied fetoplacental and maternal renin production in controls and subjects with pregnancy-induced hypertension. We measured total, active, and inactive (pro-) renin in maternal serum, fetal arterial and venous blood, and chorion homogenate in eight normotensive term patients and 18 patients with pregnancy-induced hypertension. No differences in active or prorenin were found in maternal blood from normal women or patients with pregnancy-induced hypertension. In contrast, fetal artery and vein, as well as chorionic tissue, contained significantly higher active renin in pregnancy-induced hypertension compared with normal subjects. No difference in fetal or chorionic prorenin was seen in the two groups. Thus active to total renin ratio was higher in the fetus and chorion of subjects with pregnancy-induced hypertension, which suggests enhanced active renin production. These results suggest that pregnancy-induced hypertension is associated with increased activity of the renin-angiotensin system in the fetoplacental unit, which is not reflected in the maternal circulation. This may be an attempt by the fetus and chorionic membranes to maintain vascular homeostasis in the face of altered uteroplacental blood flow. (AM J OBSTET GYNECOL 1987;157:363-7.)

Key words: Renin, pregnancy-induced hypertension

Decreased uteroplacental blood flow caused by vasospasm and vaso-occlusion may contribute to the pathogenesis of pregnancy-induced hypertension.[1] In general, this results in a growth-retarded fetus and smaller placenta in women with pregnancy-induced hypertension compared with normal pregnant women.[2] The vasospasm may be produced by increased circulating levels of a vasoconstrictor substance, which has been detected in the plasma of patients with pregnancy-induced hypertension.[3] However, the nature of this substance is unknown. Plasma renin activity is similar to or slightly lower in patients with pregnancy-induced hypertension compared with normal pregnant women, whereas angiotensin II levels have been reported to be slightly higher in pregnancy-induced hypertension.[4, 5] In pregnant women the chorionic membrane and uterus, as well as the fetus, can produce and secrete renin. Because of the impaired uteroplacental blood flow in pregnancy-induced hypertension, we sought to determine whether alterations in fetoplacental renin production occur in this disorder.

From the Division of Maternal Fetal Medicine, Department of Obstetrics and Gynecology, and the Section of Endocrinology, Department of Medicine, University of Southern California School of Medicine and Women's Hospital, Los Angeles County/University of Southern California Medical Center.
Supported by National Institutes of Health Grant AM 30254 and a grant from the American Heart Association, Florida affiliate.
Received for publication December 1, 1986; revised March 24, 1987; accepted April 27, 1987.
Presented in part at the Sixty-eighth Annual Meeting of the Endocrine Society, Anaheim, California, June 25-27, 1986, and in part at the Seventh Annual Meeting of The Society of Perinatal Obstetricians, Lake Buena Vista, Florida, February 5-7, 1987.
Reprint requests: Willa A. Hsueh, M.D., Department of Medicine, LAC/USC Medical Center, 2025 Zonal Ave., Los Angeles, CA 90033.
**Dr. Hsueh is the recipient of National Institutes of Health Research Career Development Award AM01035.*

Material and methods

In eight normotensive and 18 term laboring patients with pregnancy-induced hypertension, we obtained maternal venous blood, fetal arterial and venous cord

Table I. Clinical parameters and renin levels in control and pregnancy-induced hypertension subjects

	Control (n = 8)	*Pregnancy-induced hypertension (n = 18)*
Maternal age (yr)	25 ± 2	25 ± 2
MAP (mm Hg)	76 ± 3	113 ± 3
Gestational age (wk)	39 ± 1	39 ± 1
Length of labor (hr)	11 ± 2	16 ± 2
Birth weight (gm)	3338 ± 122	3186 ± 160
Maternal blood		
Active renin (ng AI/ml/hr)	5.4 ± 1.5	6.6 ± 1.2
Prorenin (ng AI/ml/hr)	96 ± 20	144 ± 23
Active:total renin ratio	0.06 ± 0.01	0.05 ± 0.01

MAP = Mean arterial pressure; AI = angiotensin I.

blood, and chorion. Pregnancy-induced hypertension was defined as a hypertensive disorder of pregnancy occurring after 20 weeks of gestation with a blood pressure of 140/90 mm Hg or greater on two occasions 6 hours apart or an increase of 30 mm Hg systolic or 15 mm Hg diastolic over first-trimester blood pressure. The hypertension was associated with significant proteinuria (1+ or greater) and edema. Labor was induced in none of the normotensive and six of the hypertensive subjects. All of the hypertensive subjects received $MgSO_4$, a 4 gm loading dose followed by an infusion rate of 2 gm/hr. Three of the hypertensive subjects received either methyldopa (Aldomet) or hydralazine hydrochloride (Apresoline) during the third trimester. Two normal control subjects were delivered vaginally, while six required cesarean section. Twelve subjects with pregnancy-induced hypertension delivered vaginally, and six had cesarean section. Informed consent was signed by all patients before the study. The blood samples for renin measurements and other studies were chilled and centrifuged within 4 hours after collection, and the plasma was quickly frozen to prevent inadvertent activation of plasma renin.[6] They were stored at −20° C in a nonfrost-free freezer. Clinical laboratory data included blood urea nitrogen, serum creatinine, sodium, potassium, and uric acid, which were determined by autoanalyzer.

Active renin concentration was measured by radioimmunoassay of angiotensin I after incubation of samples with sheep angiotensinogen at 37° C, pH 7.5, in the presence of angiotensinase inhibitors.[7] Total renin was measured by acid treatment of plasma,[7] which involved dialysis against pH 3.3, 0.05 mol/L glycine buffer at 4° C for 18 hours, and then dialysis against pH 7.5, 0.05 mol/L phosphate buffer under the same conditions. Inactive (pro-) renin concentration was calculated as the difference between total and active renin concentrations. The interassay coefficients of variation of active and prorenin measurements are 6% and 15%, respectively. Measurements were compared in the two groups by means of *t* test analysis, and significance was considered at the $p < 0.05$ level.

Table II. Maternal electrolytes and renal function

	Control (n = 8)	*PIH (n = 18)*	*p value*
Na (mEq/L)	138 ± 1	139 ± 1	NS
K (mEq/L)	4.1 ± 0.1	4.0 ± 0.1	NS
BUN (mg/dl)	7.6 ± 1.8	11.6 ± 2.1	<0.05
Creatinine (mg/dl)	0.6 ± 0.1	0.9 ± 0.1	<0.05
Uric acid (mg/dl)	4.1 ± 0.3	6.8 ± 0.6	<0.05

PIH = Pregnancy-induced hypertension; BUN = blood urea nitrogen.

Chorionic membrane was homogenized at 4° C in 0.25 mol/L phosphate buffer (1:2, v/v), containing 10 mmol/L ethylenediaminetetracetate, 2 mmol/L phenylmethylsulfonyl fluoride, 2 mmol/L 8-hydroxyquinoline, 2 mmol/L benzamidine, and 20,000 kallikrein inhibitor units/L aprotinin.[7] After centrifugation of the homogenate at 10,000 rpm for 30 minutes, the supernate was removed and frozen at −20° C until assay.

Results

Clinical parameters in both groups of patients are given in Table I. There were no differences in normotensive subjects and those with pregnancy-induced hypertension with regard to maternal age, gestational age, length of labor, or infant birth weight. The mean arterial pressure was significantly higher in subjects with pregnancy-induced hypertension compared with normal pregnant women. Maternal electrolytes and renal function in both groups are shown in Table II. There were no significant differences between the mean sodium and potassium levels between the two groups, whereas subjects with pregnancy-induced hypertension had significantly higher blood urea nitrogen, creatinine, and uric acid levels compared with control subjects ($p < 0.05$; Table II).

Maternal venous blood. There was no significant difference between active renin, inactive renin, and ratio of active to total renin in subjects with pregnancy-induced hypertension compared with control subjects (Table I).

Fetal arterial blood. Plasma active and inactive renin concentration in control subjects were 10.4 ± 2.4 and 65.7 ± 9.5 ng angiotensin I/ml/hr, respectively, whereas these levels were 35.7 ± 7.8 and 71.4 ± 14.0 ng angiotensin I/ml/hr, respectively, in patients with pregnancy-induced hypertension. Inactive renin

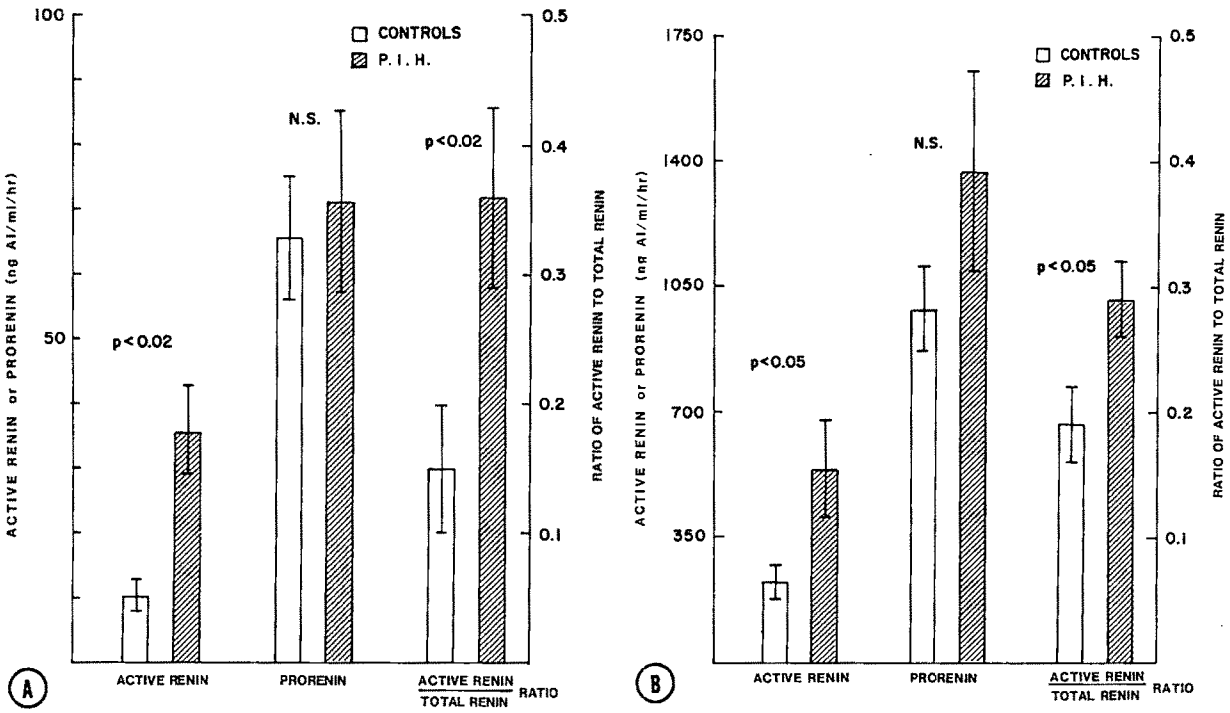


Fig. 1. Active renin and prorenin levels and active to total renin ratio in fetal arterial blood and chorion homogenate from control subjects and patients with pregnancy-induced hypertension. There were no differences between prorenin levels in fetal arterial blood (**A**) or chorion homogenate (**B**) of control subjects compared with subjects with pregnancy-induced hypertension whereas the active renin levels and the percentage of active renin were significantly higher in subjects with pregnancy-induced hypertension.

levels were not statistically different in the two groups, but samples from subjects with pregnancy-induced hypertension had significantly higher ($p < 0.025$; Fig. 1, *A*) active and total renin than did control subjects. Active to total renin ratio in subjects with pregnancy-induced hypertension (0.30 ± 0.07) was more than twice that seen in controls (0.15 ± 0.05, $p < 0.025$).

Fetal venous blood. Active and inactive renin concentrations in control subjects were 10.2 ± 3.3 and 102.21 ± 13.2 ng angiotensin I/ml/hr, respectively, whereas these levels were 44.9 ± 11.1 and 87.6 ± 18.4 ng angiotensin I/ml/hr, respectively, in blood from subjects with pregnancy-induced hypertension. Similar to fetal arterial blood, inactive renin levels were no different in the fetal venous blood of the two groups, but subjects with pregnancy-induced hypertension had significantly ($p < 0.025$) higher active and total renin than did control subjects. Active to total renin ratio in subjects with pregnancy-induced hypertension (0.30 ± 0.06) was about twice that seen in control subjects (0.15 ± 0.06, $p < 0.025$). The levels of active and inactive renin in cord arterial and venous blood were similar within the same patient.

Chorion. Homogenate of the chorionic membrane obtained from subjects with pregnancy-induced hypertension contained significantly more active renin than that of normal control subjects (542 ± 136 versus 222.5 ± 45.3 ng angiotensin I/ml/hr, respectively, $p < 0.05$). The content of inactive renin, however, was not different between the two groups (1369 ± 276 versus 988 ± 122 ng angiotensin I/ml/hr respectively, NS). Thus the active to total renin ratio was significantly higher in the chorionic membrane of subjects with pregnancy-induced hypertension compared with control subjects (0.29 ± 0.03 versus 0.19 ± 0.03, $p < 0.05$, Fig. 1, *B*).

Active renin levels in cord blood or chorionic homogenate did not correlate with maternal mean diastolic pressure, amount of proteinuria, serum creatinine, blood urea nitrogen, uric acid, sodium, and potassium, or hematocrit.

Comment

The present study is consistent with previous reports that maternal active renin concentration is normal or only slightly elevated in pregnancy, whereas inactive renin is elevated two to five times the nonpregnant range.[8] Similar levels of both active and inactive renin were seen in the blood of normal pregnant women, and several investigators have previously shown that plasma renin concentration and activity are either unchanged

or slightly decreased in subjects with pregnancy-induced hypertension compared with normal pregnant women.[5] Whether these renin levels are inappropriately high for the degree of hypertension in subjects with pregnancy-induced hypertension is still controversial.

Recent studies have demonstrated that human inactive renin is prorenin, the biosynthetic precursor of active renin.[9] Late in pregnancy the uterine fetoplacental unit contributes to the elevated prorenin levels in the maternal circulation.[10] Chorionic membranes contain large quantities of inactive renin,[11, 12] and human chorionic cells in culture secrete prorenin in relatively larger quantities compared with active renin. Factors regulating chorionic cell renin production are unknown. In the fetus the juxtaglomerular apparatus is well developed by 3 months' gestation. The fetal kidney is the major source of fetal renin, since cord blood levels of plasma renin activity are low in the anephric fetus.[13] Since absolute quantities of prorenin are increased in cord blood, prorenin may be the major form of renin secreted by the fetal kidney. Alternatively, the metabolic clearance of prorenin may be decreased in the fetus.

Because of the large quantities of prorenin secreted by the chorion and the fetal kidney, the finding of increased active renin in cord blood and chorion in pregnancy-induced hypertension was unexpected. Since prorenin levels from these sources are not significantly different in normal pregnancy and pregnancy-induced hypertension, the increased active renin levels in pregnancy-induced hypertension result from enhanced production of fetal and chorionic active renin and hence total renin production rather than from inadvertent activation of prorenin in chorionic homogenate or cord blood. An increased potential for inadvertent activation of prorenin exists in pregnancy-induced hypertension because of the increased activation of clotting factors in pregnancy-induced hypertension that are capable of activating renin.[6] However, the lack of significant increases in active renin in the maternal circulation in pregnancy-induced hypertension also argues against this possibility.

Whether the rise in chorionic and cord blood active renin preceded or resulted from maternal hypertension is an intriguing question. Fetal stress, such as hemorrhage, increases plasma renin activity in lambs and ewes.[14] In the rabbit, uteroplacental ischemia induces uterine renin release, which may contribute to the regulation of maternal systemic blood pressure.[15] Studies by Ferris and Weir[16] further indicate that one function of the uteroplacental renin angiotensin system is to maintain uteroplacental blood flow. Administration of converting enzyme inhibitor to pregnant rabbits acutely decreased uteroplacental blood flow and chronically resulted in smaller fetuses and increased fetal deaths. Thus fetal stress and hypoxia caused by a decrease in uteroplacental perfusion, which is known to occur in pregnancy-induced hypertension, may activate the fetal and chorionic renin-angiotensin systems. These results are consistent with the previous data of Broughton et al.,[17] who found increased levels of angiotensin I in the cord blood of infants born to mothers with hypertension compared with normal mothers. However, a recent report by Symonds et al.[18] showed no significant difference in plasma renin activity or active renin concentration in the cord blood of infants from normotensive women compared with that of hypertensive women. The reason for the differences in results between the two studies may be related to differences in the handling of patients. In the study of Symonds et al., labor was induced in seven of 10 hypertensive patients. Whether the patients received $MgSO_4$ or other treatment is not indicated. In our study of 18 subjects with pregnancy-induced hypertension, six had induction of labor and all received $MgSO_4$. Whether induction of labor or $MgSO_4$ affects active renin production by the chorion or fetus remains to be determined. Increases in serum magnesium concentration directly enhance renal production of renin.[19] The significance of this effect on the fetal kidney seems unlikely in the present study, since maternal active renin levels were not elevated. However, renin levels in patients with pregnancy-induced hypertension may have been lower had they not received magnesium. Although both groups of patients received similar infusions of Ringer's lactated solution, it is possible that the data might not reflect the condition before labor and some of the values may have been affected by intravascular volume.

In our pregnant women with pregnancy-induced hypertension, the impact of fetal stress could not be assessed directly, since fetal outcome and birth weight were not different from that of normal pregnancies. Serum creatinine and uric acid were significantly higher in pregnancy-induced hypertension compared with normal pregnancies, which was expected.[2] However, active renin levels in cord blood or in chorion in pregnancy-induced hypertension did not correlate with maternal blood pressure, proteinuria, serum creatinine, uric acid or electrolytes, gestational age, maternal age, or length of labor. Further studies in a larger population of patients with pregnancy-induced hypertension and in patients with evidence of intrauterine growth retardation may be necessary to determine more precisely the relationship between fetal stress and renin production. Nevertheless, this is the first study to demonstrate differences in placental and fetal renin systems compared with the maternal system in pregnancy-induced hypertension. The results suggest that perhaps imbalances in fetoplacental vasoconstric-

tor and vasodilator systems may be associated with hypertension resulting from pregnancy.

REFERENCES

1. Brosens IA, Robertson WB, Dixon HG. The role of the spiral arteries in the pathogenesis of preeclampsia. Obstet Gynecol Annu 1972;1:171.
2. Roberts JM. In: Creasy RK, Resnik R, eds. Maternal fetal medicine principles and practice. Philadelphia: WB Saunders Co, 1984:703-52.
3. Gant NF, Daley GL, Chand S, et al. A study of angiotensin II pressure response throughout primigravid pregnancy. J Clin Invest 1973;52:2682.
4. Skinner SK, Lumbers ER, Symonds EM. Analysis of changes in the renin angiotensin system during pregnancy. Clin Sci 1972;42:479.
5. Chesley LC. The renin-angiotensin system in pregnancy. J Reprod Med 1975;15:179.
6. Sealey JE, Atlas SA, Laragh JH. Prorenin and other large molecular weight forms of renin. Endocr Rev 1980;1:365.
7. Hsueh WA, Carlson EJ, Dzau V. Characterization of inactive renin from human kidney and plasma. Evidence for a renal source of circulating inactive renin. J Clin Invest 1983;71:506.
8. Hsueh WA, Luetscher JA, Carlson EJ, Grislis G, Fraza E, McHargue A. Changes in active and inactive renin throughout pregnancy. J Clin Endocrinol Metab 1982; 54:1010-6.
9. Hsueh WA, Do YS, Shinagawa T, et al. Biochemical similarity of expressed human prorenin and native inactive renin. Hypertension 1986;8:(Suppl II):78-83.
10. Brar HS, Do Y, Tam H, et al. Uteroplacental unit as a source of elevated circulating "prorenin" levels in normal pregnancy. Am J Obstet Gynecol 1986;155:1223.
11. Poisner AM, Wood GW, Poisner R. Release of inactive renin from human fetal membranes and isolated trophoblasts. Clin Exp Hypertens [A] 1982; A4:2007-17.
12. Acker GM, Galen FX, Devaux C, et al. Human chorionic cells in primary culture. A model for renin biosynthesis. J Clin Endocrinol Metabl 1982;55:902.
13. Symonds EM, Furler IK. Plasma renin levels in the normal and anephric foetus at birth. Biol Neonate 1973;23: 133-8.
14. Broughton-Pipkin F, Lumbers ER, Mott JC. Factors influencing plasma renin and angiotensin II in the conscious pregnant ewe and its foetus. J Physiol (Lond) 1974; 243:619.
15. Albertini R, Seino M, Scicli AG, Carretero OA. Uteroplacental renin in regulation of blood pressure in the pregnant rabbit. Am J Physiol 1980;239 (Heart Circ Physiol 8):H226-71.
16. Ferris TF, Weir EK. Effect of captopril on uterine blood flow and prostaglandin E synthesis in the pregnant rabbit. J Clin Invest 1983;71:809-15.
17. Broughton Pipkin F, Symonds EM. Fetal renin angiotensin system in pregnancy induced hypertension. Clin Sci Mol Med 1977;52:449-56.
18. Symonds EM, Lamming GD, Craven DJ. The fetal renin-angiotensin system in pregnancy-induced hypertension. Br J Obstet Gynaecol 1984;91:3-6.
19. Wilcox CS. The effect of increasing the plasma magnesium concentration on renin release from the dog's kidney: interactions with calcium and sodium. J Physiol (Lond) 1978;284:203-17.

Digoxin-like immunoreactive substance in fetuses with and without cardiac pathology

C. P. Weiner, M.D., S. Landas, M.D., and T. J. Persoon, M.S.
Iowa City, Iowa

The concentration of digoxin-like immunoreactive substance was measured by immunoassay in umbilical venous blood from six normal and 19 fetuses with a variety of cardiac and noncardiac disorders. Fetal blood was obtained either by percutaneous umbilical blood sampling (n = 13) or at delivery (n = 12). Three women received digoxin for fetal indications. Healthy control fetuses had significantly less digoxin-like immunoreactive substance measured (mean concentration below the limit of the assay sensitivity) than was found in ill fetuses whose mothers received digoxin ($p < 0.005$). However, the fetal concentrations of immunoreactive digoxin in fetuses with a cardiac abnormality were similar whether the mother had (0.93 ± 0.4 ng/ml) or had not (1.27 ± 0.4 ng/ml) received digoxin ($p = 0.1452$). Although there was a significant negative correlation between digoxin concentration and gestational age ($R = -0.5079$, $p < 0.01$), the youngest fetuses examined were generally the sickest. The correlation with gestational age was not significant if the normal control fetuses were excluded. One fetus with a cardiac tachyarrythmia was examined during and after transplacental therapy. There was no change. It is possible that previously measured "digoxin" in "treated" fetuses represents digoxin-like immunoreactive substance and that only small amounts of maternally administered digoxin actually reach the ill fetus. Our findings suggest that a randomized trial of maternal digitalization for the treatment of fetal supraventricular tachycardia is essential prior to its acceptance as effective therapy. (Am J Obstet Gynecol 1987;157: 368-71.)

Key words: Digoxin, digoxin-like immunoreactive substance, fetal arrythmia

Since the first successful case reported in 1980,[1] transplacental pharmacologic treatment of fetal supraventricular tachycardia has become firmly entrenched in the literature and reference textbooks despite the lack of controlled trials.[2, 3] Almost without exception, digoxin has been the drug of first choice. The reported interval from initiation of drug therapy to fetal response ranges from immediately to 35 days.[4] This status as drug of choice is presumably based on multiple studies demonstrating a near 1:1 maternal-fetal distribution of maternally administered digoxin.[5-7] In light of the rather recent discovery of a digoxin-like immunoreactive substance in patients with a variety of disorders,[8-12] the absence of data from non–digoxin-exposed control fetuses may be of significance. Frustrated by repetitive failures with transplacental digoxin therapy, despite administering drug sufficient to produce maternal toxicity, we initiated an investigation 3 years ago of fetal digoxin-like immunoreactive substance concentration in both normal and abnormal fetuses whose mothers had and had not received exogenous digoxin. The results demonstrate a need for a randomized trial of maternal digitalization prior to concluding it is effective, much less the first-line treatment for fetal supraventricular tachycardia.

From the Division of Maternal-Fetal Medicine, Departments of Obstetrics and Gynecology and Pathology, University of Iowa Medical School.
Received for publication January 16, 1987; accepted April 28, 1987.
Reprint requests: Dr. C. P. Weiner, Department of Obstetrics and Gynecology, University of Iowa Hospitals and Clinics, Iowa City, IA 52242.

Subjects and methods

Twenty-five fetuses were studied: six healthy fetuses of healthy parturient women, four fetuses whose mothers had preeclampsia but who were otherwise healthy, four fetuses with either supraventricular tachycardia or cardiac failure due to structural abnormalities whose mothers received digoxin, and 11 fetuses (12 samples) with a variety of cardiac and noncardiac disorders whose mothers had not received digoxin. Fetal blood was obtained from the umbilical vein either at delivery (n = 13) or by percutaneous umbilical cord blood sampling (n = 12). Digoxin was measured by an immunoassay (Digoxin I, Abbott TDX) used according to manufacturer's instructions. The methodology remained unchanged during the study period. This assay, like other immunoassays, does not distinguish between digoxin-like immunoreactive substance and exogenously administered digoxin.[12] For our analyses we used subprograms from ABSTAT, release 4, and included analysis of variance, correlation coefficients, and *t* tests.

Table I. Digoxin levels in normal and abnormal fetuses with and without maternal digoxin therapy

Group	*Abnormality*	*Gestational age (wk)*	*Umbilical vein "digoxin" (ng/ml)*	*Maternal vein "digoxin" (ng/ml)*	*Perinatal outcome*
Control					
1	—	38	<0.2	<0.2	Alive and well
2	—	37	<0.2	<0.2	Alive and well
3	—	40	<0.2	<0.2	Alive and well
4	—	39	<0.2	<0.2	Alive and well
5	—	38	<0.2	ND	Alive and well
6	—	39	0.4	ND	Alive and well
Preeclampsia					
7	—	36	0.5	ND	Alive and well
8	—	38	0.5	ND	Alive and well
9	—	37	0.3	ND	Alive and well
10	—	35	0.7	ND	Alive and well
Maternal digoxin therapy					
11	SVT with atrial flutter; digoxin plus propranolol	37	0.5	1.2	Responded to digoxin and cardioversion after delivery
12	SVT	32	1.0	1.7	Responded to digoxin and cardioversion after delivery
13	Nonimmune hydrops associated with Ebstein's malformation	34	1.4	1.9	Neonatal death
14	SVT	24	0.8	1.8 to 2.6	Successful direct fetal therapy
No maternal digoxin					
15	Nonimmune hydrops secondary to SVT	35	1.6	ND	Tricuspid insufficiency—converted with transesophageal pacemaker

ND = Not done; SVT = supraventricular tachycardia.

continued

Results

Only one control fetus had a detectable concentration of digoxin-like immunoreactive substance is excess of the lower limit for the assay (0.2 ng/ml) (Table I). Each fetus whose mother received digoxin had measured concentrations significantly higher than those of the healthy control fetuses ($p < 0.005$). The maternal : fetal concentration ratios for the treated pairs were similar to those reported previously. However, the digoxin concentrations in fetuses whose mothers received digoxin (Cases 11 to 14) did not differ from the digoxin-like immunoreactive substance measured in those fetuses with similar problems whose mothers had not received digoxin (Cases 15 to 18), 0.93 ± 0.41 versus 1.27 ± 0.3 ng/ml, respectively ($p = 0.1452$). One fetus (Case 14) was sampled after the maternal digoxin level had ranged between 1.8 and 2.6 ng/ml for a 72-hour period. The digoxin level of this fetus was only 0.8 ng/ml. An additional fetal specimen obtained after maternal therapy had been discontinued was not significantly different. Fetuses born to women with preeclampsia demonstrated intermediate concentrations of digoxin-like immunoreactive substance (mean = 0.5 ± 0.2 ng/ml). The highest digoxin concentration was found in a fetus without antenatal exposure to exogenous digoxin. The mean digoxin-like immunoreactive substance level of the group comprising all ill fetuses not exposed to exogenous digoxin was 0.86 ± 0.4 ng/ml. There was a significant negative correlation ($R = -0.5079$, $p < 0.01$) between gestational age and digoxin concentration. However, the youngest fetuses were usually the sickest. If one excludes the healthy control fetuses from the correlation analysis, the relationship between gestational age and digoxin-like immunoreactive substance is no longer significant.

Comment

Endogenous digoxin-like immunoreactive substance has been reported in patients with clinical disorders associated with volume expansion such as renal failure, congestive heart failure, and normal pregnancy.[7-12] The substance is for all practical purposes biologically indis-

Table I—Cont'd

Group	Abnormality	Gestational age (wk)	Umbilical vein "digoxin" (ng/ml)	Maternal vein "digoxin" (ng/ml)	Perinatal outcome
16	Nonimmune hydrops secondary to SVT	29	1.1	ND	Converted after delivery with digoxin; subsequent neonatal death; multiple endocardial rhabdomyomas
17	Nonimmune hydrops; hypoplastic left heart; in utero closure of ductus arteriosus	31	1.0	ND	Neonatal death
18	Nonimmune hydrops associated with hypoplastic transverse aorta	24	1.4	ND	In utero death
19	Twin-to-twin transfusion; specimen from recipient, hematocrit = 48%	21	0.8	ND	Delivered at 36 wk after selective feticide of cotwin
20	Twin gestation with amorphic acardiac monster and viable infant	26 31	0.5 0.1	ND ND	Alive but still in hospital; congestive heart failure; massive biventricular hypertrophy; hepatosplenomegaly; polycythemia
21	Complete heart block associated with congenital lupus erythematosus	35	0.6	ND	Alive and well
22	Complete heart block associated with congenital lupus erythematosus	38	0.6	ND	Spontaneous vaginal delivery; permanent pacemaker required
23	Obstructive hydrocephalus	32	0.5	ND	Aqueductal stenosis; status post shunt
24	Unilateral multicystic, dysplastic kidney	29	0.4	ND	Undelivered
25	Hydrocephalus; fetal anemia of unknown cause	34	0.9	ND	Neonatal death

tinguishable from exogenous digoxin[15] and its measurement highly dependent on the assay technique. Concentrations of digoxin measured by immunoassay in fetuses with cardiac abnormality whose mothers were receiving digoxin were similar to concentrations in fetuses with similar abnormality but who were not exposed antenatally to exogenous digoxin. The digoxin level of the one fetus whose maternal level was >2 ng/ml was lower than that of the control group of ill fetuses and did not change after maternal therapy was discontinued. The cardiac and noncardiac disorders (and the resulting alterations in physiologic characteristics) of these fetuses were similar to those in adults with illnesses associated with increased digoxin-like immunoreactive substance. Further, we confirm a previous report that in fetuses of women with preeclampsia there is a mild increase in digoxin-like immunoreactive substance.[14] Other "stresses" both in utero and after premature delivery lead to an increase in digoxin-like immunoreactive substance.[13, 15] Previous conclusions that these elevations were a reflection of gestational age may be equally explainable on the basis of neonatal illnesses that accompany extremes of prematurity.

Investigations of transplacental passage of digoxin may be divided into two groups on the basis of methodology of assay—immunoassay and radiolabeled drug. Chan et al.[6] measured umbilical vein digoxin concentration by radioimmune assay in 11 healthy fetuses whose mothers were receiving digoxin for a maternal illness. Their finding of a high correlation between fetal and maternal plasma concentrations of digoxin, coupled with our inability to demonstrate digoxin-like immunoreactive substance in most healthy fetuses, strongly supports the premise of transplacental passage

of digoxin under those circumstances. Chan et al. also noted high placental binding of digoxin. Saarikowski[7] administered tritium-labeled digoxin to seven women about to undergo an elective pregnancy termination. He too observed the rapid transfer of digoxin and the high placental binding but also noted low cardiac receptor affinity for digoxin at this stage in gestation. No digoxin transport studies have been performed in a pregnancy involving an ill fetus.

Our studies indicate that the concentration of digoxin-like immunoreactive substance in the serum of fetuses with supraventricular tachycardia or hydrops secondary to a cardiac lesion is high enough to account for all the "digoxin" measured by immunoassay in similar fetuses whose mothers have received digoxin. We note that the initial reports of transplacental digitalization were unsuccessful.[16, 17] These observations coupled with the wide variability in time between digoxin administration and fetal response[7] have led us to conclude that little of the maternally administered digoxin actually crosses the placenta under the pathologic circumstances studied in this investigation. The reason is unknown. We speculate that enhanced digoxin binding by the placenta may occur in these abnormal pregnancies, perhaps secondary to hyperplacentosis, which often coexists. It is also possible that the ill fetus has already achieved maximal stimulation of the digoxin receptors or, as noted in the abortuses, has a relatively low affinity for digoxin. However, two of the fetuses with supraventricular tachycardia who failed to respond to transplacental digoxin in utero did respond at least partially when treated after birth. A third fetus responded to direct fetal digitalization but not transplacental therapy. In either event, our findings suggest that further study on the movement of digoxin across the placenta of an ill fetus is necessary and that a prospective, randomized trial of maternal digoxin therapy is needed before digoxin can be accepted as the treatment of first choice for fetal supraventricular tachycardia.

REFERENCES

1. Kerenyi TD, Gleicher N, Meller J. Transplacental cardioversion of intrauterine supraventricular trachycardia with digitalis. Lancet 1980;2:393.
2. Kleinman CS, Copel JA, Weinstein EM, Santulli TV, Hobbins JC. In utero diagnosis and treatment of fetal supraventricular tachycardia. Semin Perinatol 1985;9:113-29.
3. Johnson WD, Elkayam U. Cardiac glycosides and pregnancy. In: Ekayam U, Gleicher N, eds. Cardiac problems in pregnancy: diagnosis and management of maternal and fetal disease. New York: Alan R Liss, 1982:287.
4. Wiggins JW, Bowes W, Clewell W, et al. Echocardiographic diagnosis and intravenous digoxin management of fetal tachyarrythmias and congestive heart failure. Am J Dis Child 1986;140:202-4.
5. Rogers MC, Willerson JT, Goldblatt A, Smith TW. Serum digoxin concentrations in the human fetus, neonate, and infant. N Engl J Med 1972;287:1010-3.
6. Chan V, Tse TF, Wong V. Transfer of digoxin across the placenta and into breast milk. Br J Obstet Gynaecol 1978;85:605-9.
7. Saarikowski S. Placental transfer and fetal uptake of ^{3}H digoxin in humans. Br J Obstet Gynaecol 1976;83:879-84.
8. Gruber KA, Whitaker JM, Buckalew VM. Endogenous digitalis-like substance in plasma of volume-expanded dogs. Nature 1980;287:743-5.
9. Graves SW, Brown B, Valdes R. An endogenous digoxin-like substance in patients with renal impairment. Ann Intern Med 1983;99:604-8.
10. Graves SW, Valdes R, Brown BA, Knight AB, Craig HR. Endogenous digoxin-immunoreactive substance in human pregnancies. J Clin Endocrinol Metab 1984;58:748-50.
11. Gusdon JP, Buckalew VM, Hennessy JF. A digoxin-like immunoreactive substance in preeclampsia. AM J OBSTET GYNECOL 1984;150:83-5.
12. Graves SW. Endogenous digitalis-like factors. CRC Crit Rev Clin Lab Sci 1986;23:177-200.
13. Koren G, Farine D, Maresky D, et al. Significance of endogenous digoxin-like substance in infants and mothers. Clin Pharmacol Ther 1984;36:759-63.
14. Beyers AD, Odendaal HJ, Spruyt LL, Parkin DP. The possible role of endogenous digitalis-like substance in the causation of preeclampsia. S Afr Med J 1984;65:883-5.
15. Seccombe DW, Pudek MR, Whitfield MF, Jacobson BE, Wittmann BK, King JF. Perinatal changes in a digoxin-like immunoreactive substance. Pediatr Res 1984;18:1094-7.
16. Newburger JW, Keane JF. Intrauterine supraventricular tachycardia. J Pediatr 1979;95:780-6.

Scalp heat flux and its relationship to scalp blood pH of the fetus

Rudolf Rudelstorfer, M.D., Georg Simbruner, M.D., Vinay Sharma, M.B., and Herbert Janisch, M.D.

Vienna, Austria

A method for directly assessing the metabolic rate of the fetus for optimal management of labor and delivery is still being studied. Since heat, an end product of metabolism, is dissipated from the fetal surface, we measured the heat flux from the fetal scalp and related it to the pH of fetal scalp blood, a measure of metabolism. In 25 human fetuses at risk of intrapartum hypoxia, after the membranes had ruptured and the cervix dilated to >3 cm, we attached a heat flux transducer, a platelet of 2.5 cm diameter and 1 mm thickness, to the fetal scalp and recorded continuously the heat flux from then until delivery. We also obtained one sample of fetal scalp blood in all patients and two such samples in 13 patients for the analysis of pH. We found a significant correlation between heat flux measured immediatley before the scalp blood sampling (presampling values) and the pH of fetal scalp blood ($r = 0.736$; $n = 18$ presampling values; $p < 0.001$). Out of 13 fetuses with two scalp blood samples, the changes in the scalp heat flux paralleled changes in scalp blood pH in 11. We conclude that fetal scalp heat flux is related to the metabolic condition of the fetus. Measuring scalp heat flux during labor could be developed into a noninvasive method for a continuous and more direct assessment of the fetal metabolic rate. (Am J Obstet Gynecol 1987;157:372-7.)

Key words: Scalp heat flux, scalp blood pH, fetal metabolic rate, intrapartum fetal monitoring

Many techniques are available to monitor the well-being of the fetus and to identify the fetus at risk of hypoxia.[1] However, none of them, neither cardiotocography,[2, 3] invasive assessment of biochemical variables such as pH, Po_2, or Pco_2 in the scalp tissue[4] nor ultrasound determination of umbilical blood flow,[5] yield sufficient information about changes of the metabolic rate required to accurately predict fetal outcome. One common reason for the unsatisfactory performance of these methods is that they assess the metabolic rate only indirectly.

Because an individual's heat production, heat loss, and temperature changes are related directly to metabolic rate, several authors studied temperature gradients between the mother and fetus in animals and humans to improve the assessment of the fetal condition.[6-9]

With the concept in mind that heat, an end product of metabolism, has to be dissipated from the fetal surface, we measured the heat flux from the fetal scalp by means of a heat flux transducer. In a previous study we found that scalp heat flux was significantly lower in asphyxiated than in nonasphyxiated fetuses and that during the last 30 minutes before delivery heat flux values correlated well with the pH of the umbilical cord blood.[10] To study whether heat flux changes were related to changes of pH at any time before delivery, we repeatedly measured scalp heat flux and scalp blood pH in individual fetuses. Establishing such a relationship could lead to a noninvasive, easier applicable method of continuously monitoring fetal metabolic rate.

Patients and methods

Selection criteria and consent. We studied 25 women in labor with a single fetus considered to be at risk of intrapartum hypoxia. These patients were selected according to established criteria indicating an increased fetal risk either before or during labor. Risk factors present before labor started were preeclampsia, maternal age >40 years, maternal drug abuse, fetal growth retardation, postmaturity, and significant staining of amniotic fluid.[11] Risk factors during labor were abnormal heart rate patterns.[12] The study was approved by the Ethic Committee of the University of Vienna Medical School, and informed, oral consent was obtained from all mothers. Important details of the parturients and their offsprings are presented in Table I.

Protocol and apparatus. In each woman we contin-

From the Second Universitäts-Frauenklinik und Kinderklinik Wien.
Supported by grants from the Academic Senat of the University of Vienna and funds for the advancement of medical science by the Major of the City of Vienna.
Received for publication March 1, 1987; accepted April 27, 1987.
Reprint requests: Rudolf Rudelstorfer, M.D., II. Universitäts-Frauenklinik, Spitalgasse 23, A-1090 Vienna, Austria.

Table I. Clinical characteristics of the parturients and their fetuses

Characteristic	*No.*
Women	25
Primiparous	12
Multiparous	13
Age (yr)	23.1 ± 5.2
Spontanous deliveries	9
Forceps/vacuum	8
Cesarean sections	8
Axillary temperature mean (°C)	37.00 ± 0.5
Room temperature (°C)	24.6 ± 1.1
Fetuses	25
Gestational age (wk)	39.2
Weight (gm)	3271 ± 2.2
Biparietal diameter (cm)	9.3 ± 2.2
Head circumference (cm)	34 ± 0.6
1-min Apgar	7.5 ± 1.7
5-min Apgar	9.5 ± 1.6
pH umbilical artery (-log)	7.20 ± 0.8
pH umbilical vein (-log)	7.27 ± 0.1

Values are mean ± SD.

uously recorded the cardiotocogram and the fetal scalp heat flux and at least once determined the pH of fetal scalp blood and the temperature in the mother's axilla and that of the delivery room.

The fetal heart rate was recorded by means of a spiral electrode and the uterine activity by means of an external transducer, with commercially available cardiotocographs (Hewlett-Packard Co., Palo Alto, California, or Kranzbühler—Scientific Medical Systems Co., Solingen D-5650, West Germany). The fetal scalp heat flux was measured by means of a round heat flux transducer, 25 mm in diameter and 1 mm in thickness (model Ha 15-15-13[C], Thermonetics Corp., San Diego, California). These heat flux transducers, containing thermopile on a thin, heat-conductive platelet, produce a voltage output proportional to the heat flow per unit area and time. The calibration constant describing how much heat flux is required to produce 1 mV is provided by the manufacturer. We checked the calibration constant and accuracy of the heat flux transducer with a calibration device that consisted of a copper block with an electric heating coil, which is described in detail elsewhere.[13] We found that all transducers had an accuracy better than 95% (calibration heat flux versus heat flux derived from the calibration constant and voltage output) and a linear response up to at least 100 W/m^2. The voltage output of the transducer was amplified (Multitherm, Biegler Corp., Mauerbach, Austria) and recorded on either the specially adapted cardiotocograph or a Beckman polygraph R 611 (Beckman Instruments, Inc., Fullerton, California). To ensure proper amplification of the signal, a 0.5 mV signal from a standardized electrical source was fed into the apparatus (and the recorder set) to give a 5 cm pen deflection. Calibrations were performed at the beginning of each study, but when the measuring system proved to be accurate they were performed less frequently.

Table II. Frequency of attachments of heat flux transducers necessary for successful recordings

	Frequency of attachment			
Technique of attachment	*No.*	*Once*	*Twice*	*>Twice*
Introducer and glue	9	9	0	0
Amnioscope and glue	11	6	3	2
Direct, no glue	5	2	1	2

Blood from the fetal scalp was sampled as outlined by Saling[14] and then analyzed by an AVL Gascheck 845 (AVL Coop., Graz A-8010, Austria) with automatic calibration. Fetal scalp blood analysis was used in this study despite the known drawbacks of this method because at present we do not know of any better alternative to repeatedly assess the metabolic condition of the fetus. Axillary temperature of the mother and air temperature of the delivery room were measured by standard mercury thermometers.

We obtained 40 fetal scalp blood samples in the 25 patients: one in 12 patients, two in 11, and three in two.

Attachment techniques. We attached the heat flux transducer to the parieto-occipital region of the fetal scalp after rupture of amniotic membranes in patients with a known risk of fetal hypoxia, when the cervix was dilated >2.5 cm, and in the rest of patients, when the cardiotocogram signaled an emergency and fetal blood gas analysis was indicated. With the heat flux transducer properly attached, we recorded the heat flux until delivery of the fetus. Repeated scalp blood sampling and vaginal examinations were performed with the transducer in place. At every vaginal examination we checked whether the heat flux transducer was attached correctly.

The attachment procedure was modified according to the experience gained during the study. Three practical ways of attaching the heat flux transducer emerged: attachment with glue, using an amnioscope or a special introducer device, and without glue, making use of the pressure that the surrounding tissue exerts on the fetal head.

Using an amnioscope of 40 mm diameter, we dried the scalp with gauze, introduced the heat flux transducer with glue on its surface into the vagina, and attached it to the parieto-occipital region of the fetal scalp, and after removal of the amnioscope we firmly pressed the transducer onto the scalp to ensure good contact.

Because of the inconvenience of the amnioscope we

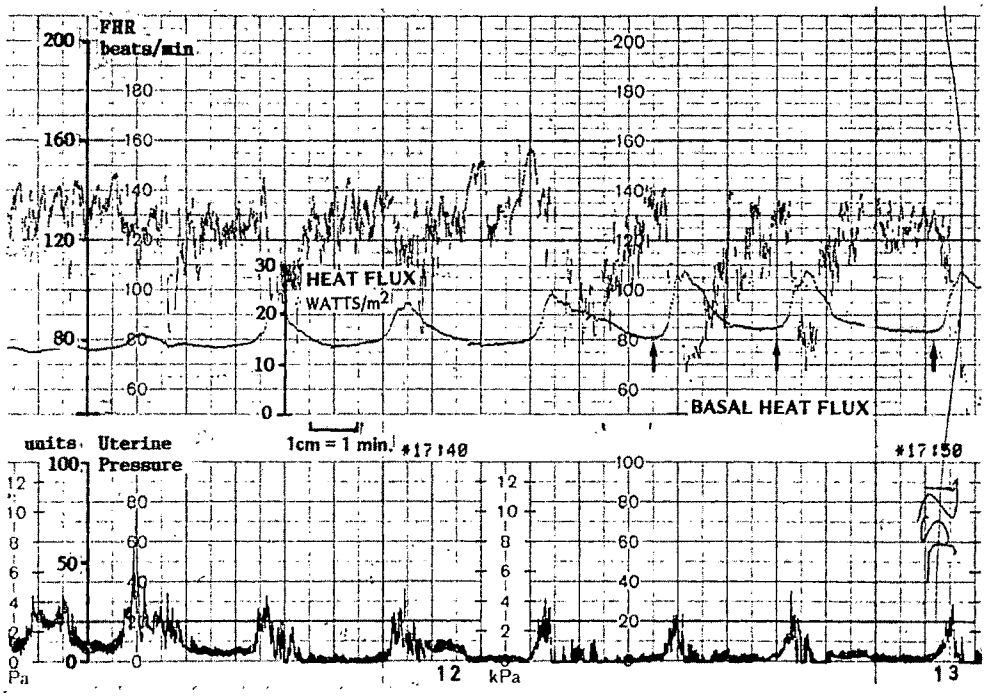


Fig. 1. Original chart of an HP-8040A cardiotocograph monitoring heart rate *(FHR)*, heat flux, and uterine pressure in a fetus with variable and overlapping declerations. Note the characteristic increase of heat flux during contractions. Basal heat flux was measured just before the onset of the next contraction.

developed a special "introducer," a lid that covers the side of the heat flux transducer where the glue is applied. It allows unhindered insertion to the site of attachment. The transducer is then slid out of its protective cover onto the fetal scalp.

For both techniques of attachment we used a water-resistant silicone adhesive (approved for use in humans; 355 Medical Adhesive, Dow Corning, Europe Inc., Brussels, Belgium). Unlike Histacryl, this adhesive absorbs heat during its binding process, has a good gluing power, and is removed easily by acetone.

In five of 25 patients we applied the heat flux transducer to the fetal scalp without glue during a vaginal examination. The transducer was inserted between the fetal scalp and the surrounding tissues during a pause of contractions and usually remained in place because of the tissue forces exerted on it. With this attachment procedure, the position of the transducer was checked more frequently by vaginal examinations.

Independent of the technique of attachment, a dislocation of the heat flux transducer was suspected when the heat flux fell to half of its previous value within a minute, the heat flux no longer varied with contractions (Fig. 1), and gentle pulling on the transducer cable was no longer met with resistance. Correct placement or dislocation was then confirmed by vaginal examination. The numbers of attachment of the heat flux transducer required for a successful recording of heat flux depended on the technique of attachment (Table II).

Analysis of data. One of the authors (V. S.), unaware of the purpose of the study, analyzed the data to avoid any bias. He identified the beginning and end of all procedures that cause artefacts in the recording of heat flux (e.g., vaginal examination, scalp blood sampling, and attachment of transducer). He then discarded these recordings and the recordings of 5 minutes after the end of such a procedure. From artefact-free recordings, the basal heat flux values before and after the fetal scalp blood sampling were then determined. Basal heat flux is the heat flux just before the onset of a contraction during which heat flux distinctly increases or decreases (Fig. 1). The onset of a contraction was identified either from the uterine pressure tracing or directly from the heat flux tracing.

Scalp heat flux values before and after the fetal scalp blood sampling (i.e., presampling and postsampling values) were used for the analysis. To study the interindividual relationship between scalp heat flux and fetal scalp blood pH, we exclusively used the one presampling value closest to delivery.

To examine the intraindividual relationship between scalp heat flux and fetal scalp blood pH, we used data pairs from patients whose pH was measured twice. If available, two presampling values and, if not, a presampling and a postsampling value were taken for analysis. To know whether presampling and postsampling heat

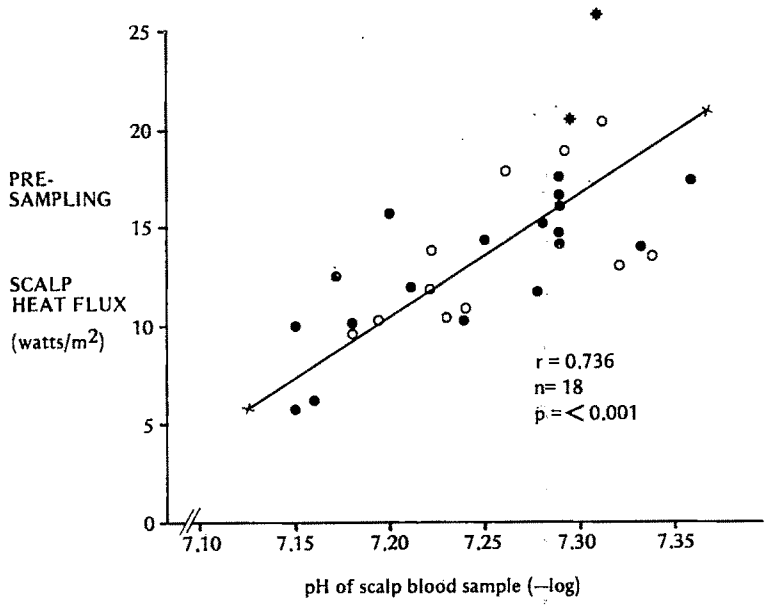


Fig. 2. Diagram shows the interindividual relationship between presampling heat flux values and scalp blood pH. Heat flux values chronologically closest (●), farther away (○), and farthest away (*) from delivery are shown.

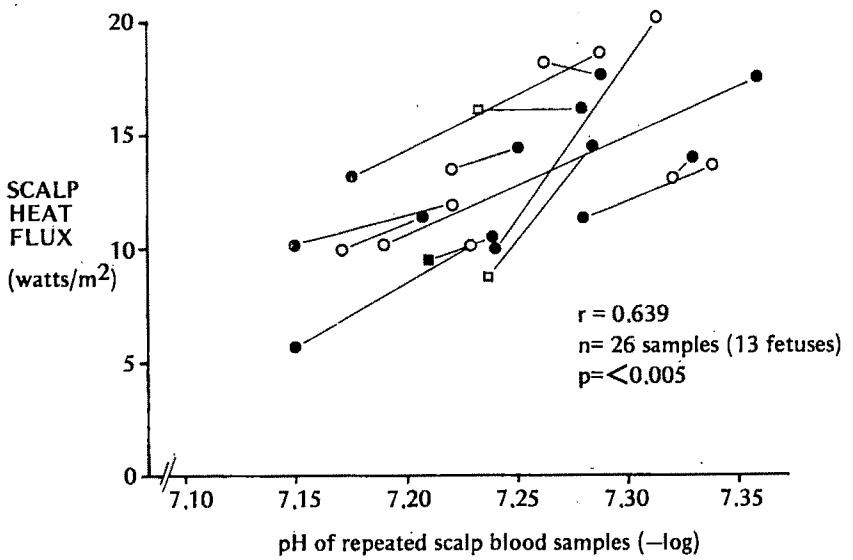


Fig. 3. Diagram shows the relationship of repeated measurements in individuals (i.e., intraindividual relationship). Presampling values chronologically closest (●) and farther away (○) from delivery and postsampling values chronologically next (■) and farther away (□) from delivery.

flux values were exchangeable, we also related both variables to each other.

Statistical analysis. Data are reported as means ± 1 SD. For statistical evaluation we performed a linear regression analysis.

Results

In 18 fetuses with presampling heat flux values, the scalp heat flux correlated significantly with the scalp blood pH ($r = 0.736$; $n = 18$; $p < 0.001$; $y = 41x - 284.5$) (Fig. 2). For each 0.1 decrease of scalp blood pH, scalp heat flux decreased approximately 5 W/m² within the range of pH 7.15 to 7.35.

Out of 13 fetuses with measurements on two occasions, changes of scalp heat flux paralleled changes of scalp blood pH in 11. In one fetus the scalp heat flux remained constant and in another it increased slightly despite a small decrease in scalp blood pH (Fig. 3).

Changes in scalp heat flux reflected increases and decreases in scalp blood pH equally well.

In 20 cases, in which presampling and postsampling heat flux values were obtained, presampling heat flux correlated statistically significantly with postsampling heat flux values ($r = 0.815$; $n = 20$; $p < 0.001$; $y = 2.556x + 0.803$). The mean difference between the two variables was 0.2 W/m² with an SD of ±0.2 W/m². Therefore, presampling and postsampling heat flux values were considered to be equally representative for actual scalp heat flux.

In 25 patients with either presampling or postsampling heat flux values the correlation between scalp blood pH and scalp heat flux was also statistically significant ($r = 0.771$; $n = 25$; $p < 0.001$; $y = 63.7x - 447.9$).

Comment

The results of this study indicate that the heat flowing from the fetal scalp is related to the metabolic condition of the fetus assessed as pH of the fetal scalp blood. These results agree with a previous study of similar nature in which the heat flux from the fetal scalp before birth was found to correlate with the pH of the umbilical arterial blood at birth.[10]

For measuring the scalp heat flux, the following conditions have to be met: First, the head has to be the presenting part and should be engaged, because a floating head does not allow sufficient pressure to be applied for the attachment. Second, the cervix has to be dilated to >3 cm for the introduction of a 25 mm diameter transducer. Smaller transducers (e.g., 10 by 20 mm) would allow attachment at a cervical dilatation <3 cm, but they are less accurate. Third, the membranes have to be ruptured for the transducer attachment with glue. However, heat flux measurements are possible with intact membranes if the head is firmly engaged in the birth channel, no glue is used, and the transducer is held in place by the tissue forces exerted on it. Fourth, some cooperation from the patient is required for the attachment of the transducer, especially when an amnioscope is used. With the special introducer, the attachment procedure does not differ essentially from the one of a spiral electrode. Fifth, attachment of the transducer with glue seems advantageous (Table II). The use of the special introducer and glue, according to our experience so far, provides satisfactory and secure attachment of the heat flux transducer with a low failure rate. However, more experience is required to accurately assess which attachment technique is optimal for which condition and which criteria other than vaginal examinations are reliably indicating the firm attachment of the transducer.

Despite a highly significant correlation between scalp heat flux and scalp blood pH, the absolute values of heat flux were somewhat scattered around the regression line (Figs. 2 and 3). Two factors could be responsible for this: heat flux is measured at only one site of the scalp and may vary with the position of the transducer on the scalp, and the temperature of the birth channel could be responsible for the variation of absolute heat flux from fetus to fetus. In the mothers studied axillary temperature ranged from 36° to 37.5° C and remained fairly constant during the time of study. The differences in the axillary temperature were large enough to be responsible for the variation in absolute heat flux on one hand but certainly small enough not to disturb the good correlation between changes in heat flux and changes of pH. For further studies we advocate measurement of the environmental temperature of the head (i.e., the temperature of the maternal soft tissue of the birth channel) and correlation of heat flux values to it.

For lack of a better method to assess the metabolic rate of the fetus repeatedly, we used the pH of the fetal scalp as a reference parameter. We were aware of the critique and limitations of this method.[14, 15] Despite all methodic drawbacks of fetal scalp blood analysis, correlations between both variables investigated were surprisingly good.

The decrease in scalp heat flux with the occurrence of metabolic acidosis (i.e., partly anaerobic metabolism) could be the result of a decreased metabolic heat production of the underlying brain or an increased circulatory heat convection out of the brain or both. Simbruner et al.[16] found that the heat flux from the head is decreased in microcephalic and hydrocephalic newborns, known for decreased cerebral heat production, and increased in children with hypoplastic left heart syndrome, known to have decreased cerebral circulation, compared with normal newborns in a similar thermal environment. Apart from metabolic heat production of and circulatory heat convection out of the brain, other factors such as scalp perfusion, reduced by acidosis, could cause the heat flux to decrease. Further studies are required to elucidate the thermal physiology of the fetal and neonatal head under pathophysiologic conditions.

From the practical point of view, monitoring the fetal scalp heat flux offers several advantages. Unlike fetal scalp blood sampling, the method is noninvasive (i.e., the scalp remains intact), safe, and technically simple and yields continuous information related to the metabolic heat production of the fetus. In contrast to cardiotocography, in which hypoxia via the autonomous nervous system alters the fetal heart rate and signals danger, in this method the measured variable is heat flux and thus is directly related to metabolism. Consequently, measuring heat flux together with cardiotocography has the potential to sort out signals of danger

from true events of hypoxia and thus to reduce the number of false positive predictions of fetal distress often followed by unnecessary surgical interventions.[1]

In conclusion, the described method and reported correlation between scalp heat flux and fetal scalp blood pH could be a first step toward a more precise system of monitoring fetal metabolic rate.

REFERENCES

1. Friedman EA. The obstetrician's dilemma: how much fetal monitoring and cesarean section is enough. N Engl J Med 1986;315:641-3.
2. Jenkins HML, Symonds EM, Kirk DL, Smith PR. Can fetal electrocardiography improve the prediction of intrapartum fetal acidosis? Br J Obstet Gynaecol 1985;93:6-12.
3. Goodlin RC. Electronic fetal monitoring as seen through the looking glass. AM J OBSTET GYNECOL 1986;155: 454-5.
4. Aarnoundse JG, Huisjes HJ, Gordon H, et al. Fetal subcutaneous scalp Po_2 and abnormal heart rate during labor. AM J OBSTET GYNECOL 1985;153:565-6.
5. Trudinger BJ, Cook CM, Jones L, Giles WB. A comparison of fetal heart rate monitoring and umbilical artery waveforms in the recognition of fetal compromise. Br J Obstet Gynaecol 1986;93:171-5.
6. Abrams R, Caton D, Curet LB, et al. Fetal brain-maternal aorta temperature differences in sheep. Am J Physiol 1969;217:1619-22.
7. Walker DW, Wood C. Temperature relationship of mother and fetus during labor. AM J OBSTET GYNECOL 1970; 107:83-7.
8. Morishima OH, Yeh MN, Niemann WH, James LS. Temperature gradient between fetus and mother as an index for assessing intrauterine fetal condition. AM J OBSTET GYNECOL 1977;141:443-8.
9. Rudelstorfer R, Tabsh K, Khoury A, et al. Heat flux and oxygen consumption of the pregnant uterus. AM J OBSTET GYNECOL 1986;154:462-70.
10. Rudelstorfer R, Simbruner G. Heat flux from the fetal scalp during labor and fetal outcome. Arch Gynecol 1983; 233:85-91.
11. Pritchard JA, MacDonald PC, Gant NF. Intrapartum surveillance of the fetus at Parkland Memorial Hospital. In: Williams obstetrics, ed 17. Norwalk, CT: Appleton-Century-Crofts, 1985:291.
12. Beard RW, Rivers RPA. Fetal asphyxia in labour. Lancet 1979;8152:1117-9.
13. Simbruner G, Weninger W, Popow C, Herholdt WJ. Reginal heat loss in newborn infants. Part I. Heat loss in healthy newborns at various environmental temperatures. S Afr Med J 1985;68:940-4.
14. Saling E. What is the role of scalp sampling in assessing "fetal distress?" J Reprod Med 1968;1:331-2.
15. Clark SL, Paul RH. Intrapartum fetal surveillance: the role of fetal scalp blood sampling. AM J OBSTET GYNECOL 1985;153:717-20.
16. Simbruner G, Weninger W, Popow C, Herholdt WJ. Reginal heat loss in newborn infants. Part II. Heat loss in newborns with various diseases—a method of assessing local metabolism and perfusion. S. Afr Med J 1985;68: 945-8.

Neonatal seizures after cesarean delivery: Higher risk with labor

William N. Spellacy, M.D., Paul Q. Peterson, M.D., Ann Winegar, M.S., and Edward J. Quilligan, M.D.

Chicago, Illinois, and Davis, California

A study was done on the occurrence of neonatal seizures in 14,367 infants delivered by cesarean section in a perinatal region in 4 years. Although seizures occurred in 1% of all of these infants, small infants weighing <2500 gm at birth had the highest frequency (>4%). Term infants delivered before labor (repeat cesarean section) had the lowest seizure rate (<0.2%), and this was a significantly lower (four times) frequency than that seen in term infants delivered by primary cesarean section. These data suggest that brain function as indexed by neonatal seizures can be adversely affected during labor. (AM J OBSTET GYNECOL 1987;157:377-9.)

From the Department of Obstetrics and Gynecology and the Center for Health Services Research, University of Illinois College of Medicine, Chicago, and the Department of Obstetrics and Gynecology, University of California School of Medicine at Davis.
Received for publication October 30, 1986; revised March 1, 1987; accepted March 13, 1987.
Reprint requests: William N. Spellacy, M.D., Department of Obstetrics and Gynecology, College of Medicine, University of Illinois, 840 S. Wood St., Chicago, IL 60612.

One of the most serious perinatal problems today is infant brain damage that may leave a child impaired for life. In some neonates an early symptom of lifetime impairment is seizures.[1] Controversy exists as to when brain damage actually occurs in infants. Some studies suggest that it may occur during pregnancy before labor begins.[2] This study was undertaken to determine

Table I. Seizures in infants weighing <2500 gm by primary and repeat cesarean sections (1982 to 1985)

	Cesarean sections			
Seizures	*Primary*	*Repeat*	*Total*	*Low risk (repeat without antepartum complications)*
No seizures				
No.	1363	441	1804	350
%	95.2	95.9	95.4	96.4
Seizures				
No.	69	19	88	13
%	4.8	4.1	4.6	3.6
Total				
No.	1432	460	1892	363
%	100.0	100.0	100.0	100.00

Probabilities not significantly different.

Table II. Seizures in infants weighing ≥2500 gm by primary and repeat cesarean sections (1982 to 1984)

	Cesearan sections			
Seizures	*Primary*	*Repeat*	*Total*	*Low risk (repeat without antepartum complications)*
No seizures				
No.	7304	5113	12417	4560
%	99.4	99.8	99.5	99.8
Seizures				
No.	47	11	58	7
%	0.6	0.2	0.5	0.2
Total				
No.	7351	5124	12475	4567
%	100.0	100.0	100.0	100.0

$p = 0.001$ (highly significant).

if there was a statistical difference between the incidence of neonatal seizures in infants delivered by elective cesarean section (no labor) and infants delivered by primary cesarean section (with labor). Infants weighing <2500 gm at birth were analyzed separately from other infants because low birth weight has such a significant impact on the occurrence of seizures, as this study will again demonstrate.[1]

Methods

The University of Illinois Perinatal Network began to electronically monitor the quality of care by abstracting all maternal and infant outpatient and hospital charts in 1982.[3] Information from approximately 17,000 deliveries per year is entered into the data base. This study includes information from 14,367 cesarean sections performed from 1982 through 1985, which represented 21.1% of the total of 68,143 deliveries during that period. In that total, 357 infants were diagnosed as having seizures (0.5%), whereas in the total cesarean section population 146 infants or 1.0% were diagnosed with seizures.

Of the total of 8783 infants delivered by primary cesarean section, 116 were diagnosed with seizures (1.3%); of the total of 5584 infants delivered by elective cesarean section, 0.5% or 30 infants were diagnosed with seizures. All analyses in the study were performed by χ^2 incorporating Likelihood Ratio, Continuity Adjusted, Mantel-Haenszel, and Fisher's exact test (one and two tail).

Results

The results of the study are shown in Tables I and II. The repeat cesarean section group was further divided into a low-risk group who had no antepartum problems such as hypertension, bleeding, and diabetes and were delivered electively without labor only because of a prior cesarean section history. Neonatal seizures were more common in small infants but the proportion of seizures was similar between primary, repeat, and low-risk repeat cesarean sections for infants whose birth weights were <2500 gm (4.8% versus 4.1% versus 3.6%; p not significant). The frequency of seizures in infants weighing >2500 gm is shown in Table II. There are significantly fewer infants with seizures in the repeat cesarean section group ($p < 0.000$); for the repeat cesarean sections the difference is three times (0.64% versus 0.21%) and for the low-risk repeat cesarean section group the difference is 4.3 times (0.64% versus 0.15%).

When comparing the differences of incidence of seizures by the two birth weight categories the percent of seizures was eight times higher in the small infants than in the large infants when primary cesarean section was the mode of delivery, although the percent of seizures was 21 times higher in small infants than in the large infants when repeat cesarean section was the mode of delivery. Thus the risk of seizure was one in 446 for term neonates delivered by cesarean section without labor, one in 156 for term infants delivered by cesarean section after onset of labor, and one in 667 for term infants of low-risk mothers who had routine repeat cesarean sections.

Comment

An understanding of the factors associated with an increased frequency of neonatal seizures may provide new information about other forms of perinatal morbidity and may allow a modification in perinatal care to reduce these forms of central nervous system damage.

In this study, as well as others, prematurity was a

significant risk factor.[1] This study also analyzed the effects of labor by studying the differences in frequency of seizures in infants delivered by elective repeat and primary cesarean sections. The repeat cesarean section group was further analyzed by comparing the subgroup that had no antepartum risk factors. In this low-risk group an elective repeat cesarean section was done and their neonates had the lowest risk of seizures (0.15%). When antepartum risk factors were present in the repeat cesarean section group, such as diabetes mellitus and hypertension, the neonatal seizure rate increased only slightly to 0.21%. Women who had labor and then underwent a cesarean section had the highest frequency of neonates with seizures (0.64%). These data suggest that labor rather than antepartum diseases is the risk factor for neonatal seizures and presumably central nervous system damage. This important finding suggests that additional studies are needed to determine when the function of the central nervous system of the infant is altered by the labor process itself.

We gratefully acknowledge the assistance of Larry Seifert, M.S., in the statistical analysis of the data. The data provided for the study belong to the members of the University of Illinois Perinatal Network and were used with their permission.

REFERENCES

1. Spellacy WN, Miller S. Neonatal seizures: an obstetrician's concern. J Perinatol 1986;6:157-60.
2. Keegan KA Jr, Waffarin F, Quilligan EJ. Obstetric characteristics and fetal heart rate patterns of infants who convulse during the newborn period. AM J OBSTET GYNECOL 1985;153:732-7.
3. Winegar A, Spellacy WN, Vidyasagar D, Peterson PQ. A system to monitor patient care in a perinatal region. AM J OBSTET GYNECOL 1983;145:39-43.

Primary cesarean section and subsequent fertility

Anita Parnes LaSala, M.D., and Alan S. Berkeley, M.D.
New York, New York

The incidence of infertility after primary cesarean section (study group) was compared to an age- and parity-matched control group who underwent vaginal delivery during 1978 at The New York Hospital. Secondary infertility occurred in 17/291 (5.8%) of the study group and 5/279 (1.8%) of the control group ($p<0.03$). Excluding those who practiced contraception and those who underwent sterilization, this difference is still significant ($p<0.02$). Excluding those patients with a history of infertility prior to the 1978 birth, there was still a trend to increased subsequent infertility in the cesarean section group although this difference did not reach statistical significance ($p<0.06$). The patients with infertility did not have a higher incidence of postpartum endomyometritis, prolonged rupture of the membranes, or abnormal placentation. The causes of the secondary infertility could not always be directly related to cesarean section; the concept of an "infertility threshold" will be discussed. (AM J OBSTET GYNECOL 1987;157:379-83.)

Key words: Cesarean section, secondary infertility, "infertility threshold"

The rate of cesarean delivery has increased dramatically over the last 15 years. Although the benefits of this trend for the unborn child have been widely debated, the maternal effects have been less thoroughly investigated. With modern blood-banking and anesthesia and with the widespread use of prophylactic antibiotics, immediate postoperative morbidity for cesarean section has decreased and death is extremely rare. Long-term maternal morbidity has been less fully examined.

The incidence of secondary infertility is unknown; its cause is diverse and in many cases unexplained. The most commonly accepted cause of secondary infertility is tubal damage and adhesions that occur after pelvic inflammatory disease[1]; the role of other causes of peritonitis is less clear-cut.[2] Three studies have failed to indicate an association between induced abortion and subsequent infertility.[3-5] Abdominal myomectomy, while frequently performed as a treatment for infertility, has also been suggested as a cause of infertility in previously fertile women.[6, 7] Endometrial ossification

From the Department of Obstetrics and Gynecology; The New York Hospital—Cornell Medical Center.
Received for publication April 8, 1986; revised March 1, 1987; accepted March 13, 1987.
Reprint requests: Alan S. Berkeley, M.D., Department of Obstetrics and Gynecology, The New York Hospital, 525 East 68th St., New York, NY 10021.

Table I. Derivation of study groups

	Cesarean section	*Vaginal delivery*
Initial No. of charts requested	502	502
Eligible, chart available	439	442
Lost to follow-up	108	127
Inadequate follow-up	40	36
Complete follow-up	291 (66.3%)	279 (63.1%)

following retained products of conception and inadvertent tubal division at the time of inguinal herniorrhaphy have also been reported as rare causes of secondary infertility.[8, 9]

The relationship of cesarean section to subsequent infertility has remained largely unexplored, although it has been postulated that postoperative endomyometritis, pelvic adhesions, and uterine cavity damage might be possible mechanisms.[10] Two recent studies have failed to document a relationship between postoperative endomyometritis and secondary infertility[11, 12]; these studies, however, used uninfected cesarean section patients as the control poulation. This study was designed to compare the incidence of infertility after cesarean section to that of an age- and parity-matched control group who underwent vaginal delivery.

Material and methods

The name and hospital number of each patient who underwent primary cesarean section at The New York Hospital in 1978 were retrieved from the daily obstetric logbook; the name and hospital number of the next age- and parity-matched patient who was delivered vaginally were also obtained. The hospital record was requested for all patients; outpatient records were reviewed and personal contact was attempted in all patients whose fertility status was still unclear after review of the inpatient chart.

Patients were considered ineligible for the study if their hospital records were unavailable or incomplete or if they underwent sterilization during the same hospitalization. Charts were reviewed for demographic data, labor and delivery events, postpartum course for mother and infant, and any history of previous infertility; the chart was also searched for any subsequent admission or information regarding future reproductive events. Even if the hospital chart was available and complete, a patient was considered "lost to follow-up" unless there was a contact with reproductive information more than 3 years after delivery; patients with a reproductive event at any time after the 1978 delivery were considered to be evaluable. All subsequent infertility was documented by medical record or personal contact with the patient by one of the authors (A. P. L.). These patients had not conceived as of January 1984 and had been trying to do so for at least 2 years.

Table II. Differences in incidence of postpartum endomyometritis between those patients lost to follow-up and those in both the study and control groups

Group	*Endomyometritis*		*Significance*
	n	%	
Cesarean section (study group)	81/291	27.9	$p < 0.005$
Cesarean section (lost to follow-up)	66/148	44.6	
Vaginal delivery (control group)	2/279	0.7	$p < 0.005$
Vaginal delivery (lost to follow-up)	9/163	5.5	

Standard febrile morbidity was defined as the presence of a temperature > 38° on at least two occasions 6 hours apart more than 24 hours after delivery. Endomyometritis was defined as the presence of standard febrile morbidity, administration of antibiotics, diagnosis documented on the chart, and absence of any other source of fever.

The χ^2 analysis with Yates' correction was used to test the significance of the data.

Results

The 502 patients who underwent primary cesarean section at The New York Hospital in 1978 constituted the study group; a like number of patients who underwent vaginal delivery constituted the control group. Table I demonstrates how many patients in each group were ineligible, were lost to follow-up, or had inadequate follow-up. Complete follow-up was available for 291/439 (66.3%) of the study group and 279/442 (63.1%) of the control group; this meant that they either had an intrauterine pregnancy at some time since delivery, were using birth control at least through 1981, or had at least 2 years of secondary infertility.

There was no significant difference between those patients lost to follow-up and those who were evaluable in terms of age, race, parity, socioeconomic status, indication for cesarean section, type of vaginal delivery, or duration of previous infertility. There was, however, a significantly higher incidence of postpartum endomyometritis in those patients lost to follow-up (Table II).

Demographic data for the two groups are presented in Table III. Those who underwent cesarean section

Table III. Demographic data for study and control groups

	Cesarean section (n = 291)		*Vaginal delivery (n = 279)*	
	n	%	*n*	%
Mean age (yr)	30		28	
Private service	238	81.8*	199	71.3*
Race				
White	222	76.3	198	71.0
Black	37	12.7	45	16.1
Hispanic and other	32	11.0	36	12.9
Incidence of primiparity	246	84.5†	217	77.8†
Incidence of previous infertility	21	7.2†	8	2.9†

*$p < 0.01$.
†$p < 0.05$.

Table IV. Subsequent reproductive outcome in study and control groups

Outcome	*Cesarean section (n = 291)*	*Vaginal delivery (n = 279)*
Viable infant	171	183
Sterilization*	7	3
Contraception*	64	52
Elective abortion†	19	26
Spontaneous abortion†	13	10
Ectopic pregnancy†	1	1
Infertility	16	4

*No intervening pregnancy.
†Only reproductive event.

had a higher incidence of previous infertility and primiparity than those who underwent vaginal delivery ($p<0.05$) and were more likely to be private patients ($p<0.01$).

Subsequent reproductive outcome is documented in Table IV. For statistical purposes those patients whose only reproductive event was an ectopic pregnancy were classified as infertile. Therefore the incidence of infertility was 17/291 (5.8%) in the study group and 5/279 (1.8%) in the control group, a significant difference ($p<0.03$). If one excludes those patients using contraception or having undergone sterilization and includes only those at risk for pregnancy, the incidence of infertility was 17/220 (7.7%) in the study group and 5/224 (2.2%) in the control group, also a significant difference ($p<0.02$). If one includes those patients who had only spontaneous abortions in the infertility category, the incidence of infertility becomes 30/220 (13.6%) in the study group at risk for pregnancy and 15/224 (6.7%) in the control group, also significantly different ($p<0.02$).

Table V compares the incidence of certain factors that might be related to subsequent infertility between those patients with subsequent infertility and those without in both groups; this includes history of previous infertility, occurrence of infection, length of ruptured membranes, and indication for cesarean section. Significantly more patients who had infertility after cesarean section had a history of infertility prior to that delivery compared with those who did not have infertility after cesarean section ($p<0.05$). If all patients with a history of previous infertility are excluded from our analysis, 13/204 (6.4%) of those who underwent cesarean section developed secondary infertility compared with 5/224 (2.2%) of those who had a vaginal delivery; a stong trend for secondary infertility after cesarean section persists although it does not quite reach statistical significance ($p<0.06$).

Table VI documents the causes of the subsequent infertility, if known, in both groups.

Comment

Despite the difficulties inherent in obtaining long-term follow-up in a mobile, urban population, a substantial number of patients (64.7%) were completely evaluable by our criteria. Those lost to follow-up were different from those included in the study in only one respect, namely, their higher incidence of endomyometritis. Although suggested as a possible cause of subsequent infertility, the effect of postpartum endomyometritis has been discounted both by our own data (those with subsequent infertility after cesarean section had a lower rate of postpartum endomyometritis than those with established fertility after cesarean section) and by two recent studies.[11, 12] In fact, if postpartum endomyometritis were a cause of subsequent infertility, then our study is underestimating post–cesarean section infertility because the number of cesarean section patients lost to follow-up includes many more with postpartum endomyometritis than those lost to follow-up in the vaginal delivery group. Therefore the conclusions drawn from our study population are valid.

Secondary infertility was found significantly more frequently in those patients who underwent cesarean section. The patients with infertility did not have a higher incidence of postpartum endomyometritis, prolonged rupture of the membranes, or placental abnormalities when compared with those without subsequent infertility. However, in the control group the absolute incidence of secondary infertility was extremely low (2.2%); on a population basis the 7.7% reported for the study group would, if confirmed, represent a dramatic increase in infertility related to cesarean section. Of further interest, the rate of secondary infertility for our entire population was 22/484 (4.5%). However, our cri-

Table V. Incidence of factors that might be related to subsequent fertility in fertile and infertile patients in both study and control groups

Factor	*Cesarean section*		*Vaginal delivery*	
	Subsequently infertile (n = 17) (%)	*Subsequently fertile (n = 274) (%)*	*Subsequently infertile (n = 5) (%)*	*Subsequently fertile (n = 274) (%)*
Previous infertility	23.5*	6.2*	0	2.9
Postpartum endomyometritis	17.6	28.5	0	0.7
Other infection†	17.6	8.4	0	1.5
Intact membranes	35.3	24.5	0	0
Membranes ruptured				
1-6 hr	23.5	33.6	80	61.1
7-24 hr	41.2	36.5	20	34.3
>24 hr	0	5.5	0	4.6
Indication for cesarean section				
Cephalopelvic disproportion	52.9	46.0		
Fetal distress	17.6	19.7		
Abnormal presentation	11.8	21.5		
Abnormal placentation	0	6.2		
Multiple gestation	0	1.5		
Maternal indications	17.6	8.8		

*$p < 0.05$.

†Urinary tract infection, mastitis, wound infection.

Table VI. Causes of secondary infertility in study and control groups

Cause	*Cesarean section (n = 17)*	*Vaginal delivery (n = 5)*
Asherman's syndrome	2	0
Male factor	4	1
Tubal factors	2	2
Ovulatory dysfunction	2	0
Unknown, evaluation in progress	7	2

teria and methodology probably cause us to underestimate the incidence of secondary infertility in all groups. This is because we used 2 years rather than the more standard 1 year as our definition of infertility and because infertility patients are less likely than maternity patients to be hospitalized, making them more likely to be lost to follow-up.

Infertility can be a multifactorial problem. It is possible that woman with one or more minor abnormalities of reproductive function can still become pregnant if the sum of the effects of these abnormalities falls below some "infertility threshold." The added stress of a cesarean section (peritubal adhesions or intrauterine injury) may push these women over the threshold and result in subsequent infertility. One could argue that patients with a history of previous infertility should be excluded from calculations of the rate of secondary infertility after cesarean section because it is difficult to assess how much of this secondary infertility can be attributed to the mode of delivery and how much to a preexisting fertility problem. However, we do not believe that exclusion of these patients is either necessary or appropriate, because the definition of "previous infertility" used in this study is very broad. We included in this category any patient who ever had an infertility workup, with or without a diagnosis being established.

Of the 17 patients with infertility after cesarean section, four are said to have previous infertility. Two of these women started a fertility evaluation, underwent normal hysterosalpingography, had no further workup, and conceived. One woman stated that she had a "hormonal imbalance" discovered after a workup that included normal semen analysis and hysterosalpingography; she was treated with a progestational agent for 5 days and conceived. This "therapy" was repeated, unsuccessfully, after cesarean section when she again complained of infertility. The last patient conceived with the help of clomiphene citrate and insemination with sperm of her husband. After the cesarean section, this therapy, as well as human menopausal gonadotropin therapy, was tried without success. In reality, although included in our calculations, the first three patients had no cause of infertility identified before cesarean section. The fourth patient had an ovulatory problem successfully treated with medical therapy prior to operation that failed to respond to the same, and more intensive, therapy after operation; this suggests that an additional factor had come into play. Any increase in the rate of secondary infertility caused by the inclusion of these patients in our statistics only serves to confirm our thesis that cesarean delivery causes patients with marginal fertility to become infertile.

This concept of a threshold is important because only

four of our 17 study patients with infertility had verified tubal or intrauterine disease as the sole cause of infertility. Most authorities would accept that these abnormalities resulted from operation. The other 13 patients had a cause that either was not clearly directly related to cesarean section or was as yet unknown. It is in these patients that surgically related subclinical injury may have an additive effect to other nonsurgical infertility factors that may have knowingly or unknowingly been present before the cesarean section. This threshold concept may explain the paradox of increased infertility after cesarean section unexplained by obvious postsurgical morbidity.

Asherman's syndrome is a rare occurrence after cesarean section without curettage.[13, 14] Nevertheless, two of the patients in our study group suffered this complication and required hysteroscopic division of intrauterine synechiae. One has subsequently had an ectopic pregnancy and the other has as yet failed to conceive.

Two recent epidemiologic studies addressed the issue of the effect of cesarean section on subsequent fertility. Zdeb et al.[15] demonstrated an 11% decrease in subsequent pregnancies in women who had undergone primary cesarean section as compared with those who had a vaginal delivery. It could not be determined from this study whether this was a voluntary difference. Hemminki et al.[16] came to a similar conclusion; however, patients undergoing surgical sterilization were not excluded from the data, and there were more of these patients in the cesarean section group. Our study addresses both these issues by excluding women who were practicing contraception or had undergone sterilization. We suggest that the difference in fertility is not voluntary; there is a significantly higher incidence of infertility after cesarean section among women desiring pregnancy. If confirmed by future investigations, this information will have to be added to the other components of the risk-benefit equation for primary cesarean section.

REFERENCES

1. Walton SM, Mati JKG. An evaluation of secondary infertility in Kenya. S Afr Med J 1976; 53:310.
2. Puri P, Guiney EJ, O'Donnell B, et al. Effects of perforated appendicitis in girls on subsequent infertility. Br Med J 1984; 288:25.
3. Hogue CJ, Cates W, Tietze C. The effects of induced abortion on subsequent reproduction. Epidemiol Rev 1982; 4:66.
4. Daling JR, Spadoni LR, Emanuel I. Role of induced abortion in secondary infertility. Obstet Gynecol 1981; 57:59.
5. Stubblefield PG, Monson RR, Schoenbaum SC. Fertility after induced abortion: a prospective follow-up study. Obstet Gynecol 1984; 63:186.
6. Babaknia A, Rock JA, Jones HW. Pregnancy success following abdominal myomectomy for infertility. Fertil Steril 1978; 30:644.
7. Berkeley A, Polan ML, DeCherney AH. Abdominal myomectomy and subsequent infertility. Surg Gynecol Obstet 1983; 156:319.
8. Swanson JA, Chapler FK. Infertility as a consequence of bilateral herniorrhaphies. Fertil Steril 1977; 28:118.
9. Dutt S. Endometrial ossification associated with secondary infertility. Br J Obstet Gynaecol 1978; 85:787.
10. Kemper TT, Trimbos B, Van Hall E. Etiologic factors in tubal infertility. Fertil Steril 1982; 37:384.
11. Valenzuela G. Fertility following cesarean section endoparametritis. AM J OBSTET GYNECOL 1984; 149:231.
12. Hurry DJ, Larsen B, Charles D. Effects of post-Caesarean section febrile morbidity on subsequent fertility. Obstet Gynecol 1984; 64:256.
13. Taylor PJ, Cumming DC, Hill PJ. Significance of intrauterine adhesions detected hysteroscopically in eumenorrheic infertile women and role of antecedent curettage in their formation. AM J OBSTET GYNECOL 1981; 139:239.
14. Buttram VC, Turati G. Uterine synechiae: variations in severity and some conditions which may be conductive to severe adhesions. Int J Fertil 1977; 22:98.
15. Zdeb MS, Therriault GD, Logrillo VM. Frequency, spacing, and outcome of pregnancies subsequent to primary cesarean childbirth. AM J OBSTET GYNECOL 1984;150: 205.
16. Hemminki E, Graubard BI, Hoffman HJ, et al. Ceasarean section and subsequent fertility: results from the 1982 National Survey of Family Growth. Fertil Steril 1985; 43:520.

A study on intrauterine fetal resuscitation with terbutaline

Michael S. Patriarco, D.O., Bruce M. Viechnicki, M.D., Thomas A. Hutchinson, M.D., Stephen K. Klasko, M.D., and Sze-Ya Yeh, M.D.

Allentown, Pennsylvania

A randomized study on the effect of terbutaline on fetal distress was carried out in 20 patients who showed evidence of ominous fetal heart rate patterns and fetal scalp blood pH values of <7.25. Of those, 11 received terbutaline (study group) and nine did not (control group). There was a significant improvement in the acid-base status of the fetus in the study group compared with those in the control group ($p < 0.01$). No significant maternal or fetal morbidity occurred in the study group. Apgar scores at 1 minute were 7 or greater in 10 of the 11 study subjects whereas only four of the nine control subjects had a score of 7 or greater. These results suggest that terbutaline may become a useful agent in the treatment of intrauterine fetal distress. (Am J Obstet Gynecol 1987;157:384-7.)

Key words: Terbutaline, fetal distress, fetal resuscitation

Recent advances in continuous electronic fetal heart rate monitoring and fetal capillary blood pH measurements have facilitated the detection of fetal distress in utero; however, the treatment of fetal distress has remained relatively unchanged during the last two decades. Acute fetal distress, when indicated by ominous fetal heart rate patterns or acidotic fetal capillary blood, requires prompt intervention. If no relief occurs with conventional methods (i.e., changing the maternal position, oxygen administration, and discontinuation of oxytocin if it is in use), immediate delivery should be considered by either cesarean section or rapid operative vaginal delivery.

When immediate cesarean delivery is indicated, the time interval required to prepare for the surgery may be too long for an already-compromised fetus. Therefore, any intermediate treatment that could prevent further deterioration of the fetal condition would be very useful.

Caldeyro-Barcia et al.[1] first described the potential use of a pharmacologic agent for intrauterine resuscitation. In their study a β-sympathomimetic agent, metaproterenol sulfate, was used. By decreasing uterine activity through β-sympathomimetic activity, uterine perfusion to the fetus could be increased, thereby improving its acid-base status. Pharmacologically, the advantages of using this agent for intrauterine resuscitation are manyfold: first, the rapid pharmacologic effect demonstrated by this agent; second, increased maternal venous return, stroke volume, and cardiac output as a result of its vasodilating effect (this increase results in improvement of the uteroplacental blood flow[2, 3]); third, increased cardiac output and uteroplacental blood flow caused by maternal tachycardia and sympathetic activity[2, 3]; fourth, increased uteroplacental blood flow caused by the β-sympathomimetic agent[4-8]; and fifth, enhanced transfer of glucose from the mother of the fetus because of maternal hyperglycemia caused by this agent.[3]

Because of the limited number of published articles studying the issue of intrauterine resuscitation, this prospective, randomized study was undertaken to evaluate terbutaline, a β-sympathomimetic agent, for the management of intrapartum fetal distress.

Material and methods

This study was carried out in the labor and delivery suite at the Allentown Hospital from January 1984 to December 1985. When patients in labor showed signs of fetal distress as indicated by repetitive late decelerations or severe variable decelerations with internal fetal heart rate monitoring, they were managed initially with conventional methods (i.e., administrating oxygen to the mother by mask, changing maternal positions, placing her in the Trendelenburg position when needed, and discontinuing oxytocin infusion if it was in use). If fetal heart rate deceleration patterns persisted despite the above measures, a fetal scalp blood sample was obtained, and the pH was measured with a blood gas analyzer (Instrumentation Laboratory, Inc., Lexington, Mass.). If the pH value was below 7.25, which indicated fetal distress,[9] the patient was selected for the study after a proper informed consent was obtained. She was then randomly assigned to the study or the control group by randomly generated numbers. While preparations were being made for delivery the patients in the study group were given 0.25 mg terbutaline sulfate injection subcutaneously, and those in

From the Department of Obstetrics and Gynecology, Allentown Hospital.

Received for publication May 17, 1986; accepted March 13, 1987.

Reprint requests: Michael S. Patriarco, D.O., the Allentown Hospital, 17th and Chew Sts., Allentown, PA 18102.

Table I. Demographic data of patients in the study group and the control group

	Study group (n = 11)	*Control group (n = 9)*
Maternal age (yr)*	23.0 ± 1.3	26.0 ± 1.8
Gestational age (wk)*	39.7 ± 0.5	39.2 ± 1.8
Parity		
Nulliparous	5	4
Multiparous	6	5
Complications		
None	3	2
Postdatism	2	2
IUGR	2	1
Hypertension	0	1
Meconium passage	3	2
Prematurity	0	1
Oligohydramnios	1	0
Anesthetic for delivery		
General	9	6
Regional	2	3

IUGR, Intrauterine growth retardation.
*Mean ± SEM.

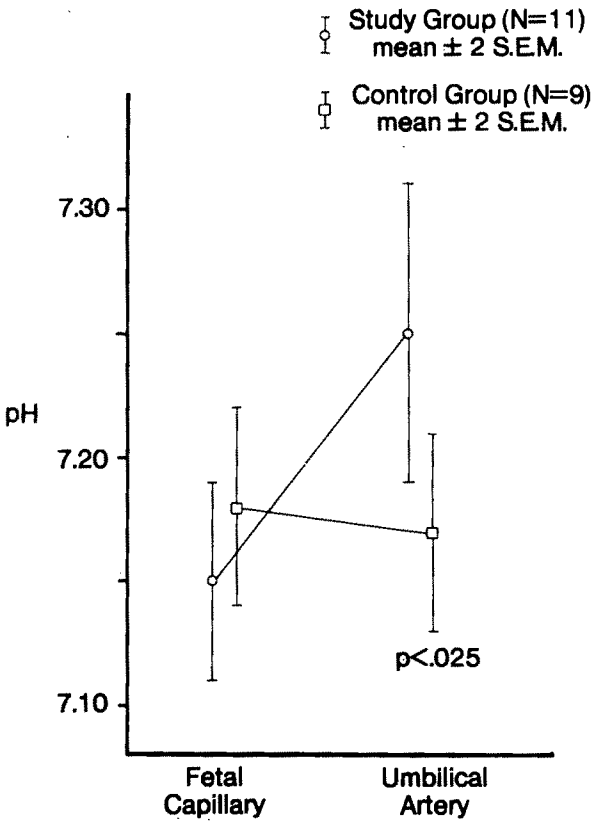


Fig. 1. pH change in the study and control groups.

the control group received no medication. Fetal heart rate monitoring was continued in both groups until the infant was delivered. At the time of delivery, blood samples were obtained from the umbilical artery in heparinized syringes for pH determinations. Immediately after delivery, all neonates were evaluated by a pediatrician or neonatologist in the delivery room and Apgar scores were given.

Twenty pregnant patients were selected for this study. Eleven patients were randomized as the study group and nine as the control group. A separate t test was used to compare the pH values and a χ^2 test was used to compare the Apgar scores in two groups. A p value <0.05 was used to indicate significant differences.

Seven additional cases are presented for discussion. They were not included in the study because a fetal scalp blood sample was not obtained before administration of terbutaline because of urgent situations that required immediate intervention.

Results

Table I lists the demographic data in both groups. These include the maternal age, parity, gestational age, complications of pregnancy, and type of anesthetic used for delivery. There was no significant difference found between the groups.

Of the 11 patients in the study group, seven demonstrated fetal distress as indicated by late decelerations whereas the remaining four patients had severe variable decelerations. After terbutaline administration, five patients demonstrated complete cessation of uterine activity whereas the remaining six patients showed diminished frequency of contractions. In all five patients in whom uterine activity subsided, no further fetal heart rate decelerations were noted. Five of the six remaining patients continued to have fetal heart rate decelerations at a lesser degree of amplitude and frequency. Only one patient did not show any improvement in the fetal heart rate deceleration patterns.

Of the nine patients in the control group, six showed repetitive late decelerations and three had severe variable decelerations. In all instances no improvement was noted in the fetal heart rate deceleration patterns or the uterine activity before delivery.

Fig. 1 and Table II summarize the changes of blood pH values in both the control and study groups. In the control group the mean fetal scalp blood pH was 7.18 with an SEM of 0.02. In the study group the mean fetal pH was 7.15 with an SEM of 0.02. The difference of means between these two groups was not significant. The mean umbilical arterial blood pH in the control group was 7.17 with an SEM of 0.02, whereas the mean umbilical artery pH of the study group was 7.25 with an SEM of 0.03. This difference was statistically significant at $p < 0.025$. In 10 of the 11 treated patients the umbilical artery pH was greater than the fetal capillary blood pH. In six of the nine patients in the control group the umbilical artery pH was actually lower than the last fetal pH value. A t test was performed to compare the mean of the fetal capillary blood pH with the

Table II. Comparison of pH values from fetal scalp blood and umbilical arterial blood between the study group and the control group

	Fetal pH		*Umbilical pH*	
	No.	*Mean ± SEM*	*Mean ± SEM*	*p Value**
Study group	11	7.15 ± 0.02	7.25 ± 0.03	<0.01
Control group	9	7.18 ± 0.02	7.17 ± 0.02	NS
p Value*		NS	<0.025	

*p Values were obtained from separate t test.

Table III. Comparison of Apgar scores between the study and the control group

	Study group (n = 11)	*Control group (n = 9)*	*p Value**
1 min Apgar score			
<7	1	4	<0.025
≥7	10	5	
5 min Apgar score			
<7	0	2	NS
≥7	11	7	

*p Values were obtained from the χ^2 test.

mean of the umbilical artery pH in both groups. The umbilical artery mean pH was found to be significantly higher than the fetal scalp blood pH in the study group ($p < 0.01$), whereas there was no statistical difference in the control group.

Ten of the 11 patients in the study group had 1-minute Apgar scores of 7 or greater. However, in the control group only four of the nine patients had 1-minute Apgar scores of 7 or greater. This difference was statistically significant at $p < 0.025$ (Table III).

There were no perinatal deaths in either group. The mean birth weight in the study group was 7 pounds 2 ounces with a range of 4 pounds 8 ¾ ounces to 9 pounds 4 ¾ ounces. The mean birth weight in the control group was 7 pounds 5 ounces with a range of 4 pounds 15 ¾ ounces to 9 pounds 2 ounces. There was no apparent difference in neonatal morbidity in either group.

There were no maternal side effects noticed as the result of terbutaline administration. A mild tachycardia was noted in patients who received medication; however, they remained symptom free at all times.

All patients in this study were delivered by cesarean section. Nine of the 11 patients in the study group were delivered under general anesthetic, one under spinal anesthetic, and one under epidural anesthetic. In the control group, six of the nine patients were delivered under general anesthetic, one under epidural anesthetic, and two under spinal anesthetic.

In the seven additional patients presented, who did not meet protocol requirements, five were delivered by cesarean section and two were delivered vaginally. Both of the patients delivered vaginally had prolonged fetal bradycardias lasting 7 minutes or longer that did not respond to oxygen administration, positional changes, or discontinuation of oxytocin. While preparing for cesarean section, terbutaline was administered subcutaneously. Within the next 5 minutes the fetal heart rate returned to normal. Both patients were allowed to continue labor and had successful vaginal deliveries. The remaining five patients had urgent situations that required immediate delivery. There was not sufficient time to obtain a scalp blood sample for pH determination before cesarean section. All patients received terbutaline before surgery. The umbilical artery blood pH was >7.25 in three of these five patients. Four of the five patients had a 1-minute Apgar score of 7 or greater.

Comment

Once detected, fetal distress has traditionally been treated by giving oxygen, changing maternal positions, or discontinuing oxytocin infusion. If there is no apparent improvement in the fetal status indicated by the fetal scalp blood pH, or the fetal heart rate tracing, prompt delivery should be considered.

In 1969 Caldeyro-Barcia et al.[1] described a potential new method for the treatment of fetal distress. In this study, metaproterenol sulfate, a β-sympathomimetic agent, was administered through a continuous intravenous infusion. In this report, fetal scalp blood samples were obtained for pH determination sequentially after the initiation of metaproterenol sulfate infusion until the fetal pH recovered to 7.30 or greater. Once the patient had recovered, the infant was delivered by cesarean section in a "resuscitated state." Most of the subsequent studies on this subject were done in foreign countries.

In this country, Arias[10] attempted to manage acute intrapartum fetal distress with terbutaline administration. Fifteen pregnant women in whom fetal distress was detected by electronic fetal heart rate monitoring who did not respond to maternal positional changes, oxygen administration, or discontinuation of oxytocin received terbutaline, 0.25 mg, subcutaneously or intra-

venously. All patients showed evidence of partial or total fetal recovery after the drug administration. However, this study did not provide a biochemical evidence of fetal recovery.

Tejani et al.[11] also used terbutaline for intrauterine fetal resuscitation. Eighteen patients were selected for the study with the criteria of fetal distress defined by fetal acidosis (fetal scalp blood pH <7.25).[9] Ten of the 18 patients received terbutaline. There was an obvious improvement of fetal heart rate patterns and fetal acid-base status when fetal scalp pH was compared with the umbilical artery pH in these treated cases. Although this was not a randomized study, there appeared to be evidence suggesting that terbutaline could be used as a resuscitative agent in utero.

Lipshitz[12] used an intravenous bolus injection of hexoprenaline, another β-sympathomimetic drug, for the treatment of fetal distress in six patients. A rapid improvement in fetal heart rate occurred on all occasions. Again, this study did not provide biochemical evidence of fetal improvement.

In our study, pH was chosen to demonstrate fetal distress because previous studies have indicated a poor correlation between ominous fetal heart rate patterns and the Apgar scores.[1] We have demonstrated a significant improvement of the acid-base status in the neonates of the study group as compared with the control group. This study also showed evidence of better neonatal condition in the study group in terms of Apgar scores. No significant maternal or fetal side effects occurred in any of the patients who received terbutaline.

The results of this study suggest that terbutaline may be useful in the management of fetal distress during labor when other conventional methods have failed. Terbutaline offers several advantages in the management of acute fetal distress. First, terbutaline seems to improve the acid-base status of the fetus immediately and therefore allows delivery of a resuscitated infant rather than a depressed infant. Second, giving terbutaline may decrease the need for immediate delivery. This may be of great benefit, especially in a hospital in which emergency anesthetic coverage is not readily available. Third, since fetal distress may be a transient phenomenon, terbutaline may assist the fetus in overcoming this transient stress and allow the patient to continue labor. Fourth, since terbutaline may decrease the urgency of delivery, additional time may be available for the patient to receive conductive anesthetic, thereby decreasing the additional risks of "crashed" cesarean section and general anesthetic.

We gratefully acknowledge the cooperative effort of the attending and nursing staff of the Allentown Hospital.

REFERENCES

1. Caldeyro-Barcia R, Magana JM, Castillo JB, et al. A new approach to the treatment of acute intrapartum fetal distress. In: Perinatal factors affecting human development. Scientific Publication No. 185. Washington, DC. Pan-American Health Organization, 1969:248-53.
2. Bienarz J, Ivancovich A, Scommegna A. Cardiac output during ritodrine treatment for premature labor. AM J OBSTET GYNECOL 1974;118:910.
3. Coleman AJ, Leary WP. Cardiovascular effects of hexoprenaline sulphate and salbutamol in man. South Afr Med J 1973;47:621.
4. Brettes JP, Renaud R, Gander R. A double-blind investigation of ritodrine on uterine blood flow during the third trimester of pregnancy. AM J OBSTET GYNECOL 1976;124:164.
5. Janisch H, Leodolter S, Reinold E. Der effekt von ritodrine auf die plazentadurch blutung. In: Baumgarten K, Wesselius-de-Casparis A, eds. Proceedings of the international symposium, the treatment of fetal risk, Baden, Austria, May 11-13, 1972. Vienna: University of Vienna (Medical School, Second Department of Obstetrics and Gynecology) 1973:51-2.
6. Akerlund M, Andersson KE, Ingemarsson I. Effects of terbutaline on myometrial activity, uterine blood flow, and lower abdominal pain in women with primary dysmenorrhea. Br J Obstet Gynaecol 1976;83:673.
7. Lippert TH, Degrandi PB, Fridrich R. Action of the uterine relaxant fenoterol on uteroplacental hemodynamics in human subjects. AM J OBSTET GYNECOL 1976;125:1093.
8. Gamissans O, Carreras M, Duran P, et al, eds. In: Proceedings of the international symposium, the treatment of fetal risk, Baden, Austria, May 11-13, 1972. Vienna: University of Vienna (Medical School, Second Department of Obstetrics and Gynecology) 1973:145-8.
9. Tejani N, Mann LI, Bhakthavathsalan A. Correlation of fetal heart rate patterns and fetal pH with neonatal outcome. Obstet Gynecol 1976;48:460.
10. Arias F. Intrauterine resuscitation with terbutaline: a method for the management of acute intrapartum fetal distress. AM J OBSTET GYNECOL 1978;131:39.
11. Tejani N, Verma UL, Chatterjee S, Mittelman S. Terbutaline in the management of acute intrapartum fetal acidosis. J Reprod Med 1983;28:857.
12. Lipshitz J. Use of a beta-sympathomimetic drug as a temporizing measure in the treatment of acute fetal distress. AM J OBSTET GYNECOL 1977;129:31.

A randomized trial of ritodrine tocolysis versus expectant management in patients with premature rupture of membranes at 25 to 30 weeks of gestation

Thomas J. Garite, M.D., Kirk A. Keegan, M.D., Roger K. Freeman, M.D., and Michael P. Nageotte, M.D.

Long Beach, California

Expectant management was compared with similar management plus ritodrine tocolysis in a randomized controlled trial in patients with premature rupture of membranes at 25 to 30 weeks of gestation. In the tocolysis group intravenously administered ritodrine was instituted at the onset of labor and then changed to the oral form if successful. Tocolysis was discontinued or not instituted after 31 weeks of gestation. Seventy-nine patients were randomized over a 4-year period, 39 in the tocolysis group and 40 in the expectant group. Twenty-three patients in the tocolysis group actually received ritodrine. No difference between the two groups was demonstrated in the interval between premature rupture of membranes and delivery or in reaching 32 weeks of gestation. No statistical difference was seen in maternal morbidity. Birth weights and gestational ages at delivery were similar between the two groups as were the incidences of neonatal morbidities caused by prematurity and infection and in the duration of neonatal hospital stays. Despite being conducted in those gestational ages in which prolongation of pregnancy might be expected to be of most benefit, no difference could be demonstrated with the addition of tocolytic therapy over expectant management alone. (Am J Obstet Gynecol 1987;157:388-93.)

Key words: Tocolysis, PROM

Premature rupture of membranes (PROM) is the most common single diagnosis leading to premature delivery and neonatal complications that require admission to a neonatal intensive care unit. Better understanding, newer antibiotics, and improved diagnostic techniques have led to fewer major maternal complications. As a consequence, in recent years there has been an increasing tendency to develop management protocols designed principally to prolong pregnancy and decrease neonatal complications from prematurity.

Premature labor is a natural consequence of PROM, since 80% to 90% of preterm patients will go into labor within a week of membrane rupture.[1] With the availability of effective tocolytic agents it would seem logical to apply this form of therapy to patients with premature labor following PROM. In the past experts have thought that tocolysis was contraindicated in such patients primarily because of the concern of increasing the likelihood of infectious morbidity and mortality.[2] However, to date there have been few data from controlled studies on which to evaluate the efficacy and safety of attempting to stop labor and delay preterm delivery in patients after PROM.

Therefore, a prospective randomized study was developed to evaluate the use of tocolysis in patients with preterm labor after PROM. Because it has been shown that tocolytic therapy is generally less effective in patients with ruptured as opposed to intact membranes, we chose to study patients with PROM in the very premature gestation, in which any benefit from prolongation of pregnancy and the consequent improvement in neonatal outcome might be expected to be most likely demonstrated.

Material and methods

All patients admitted to the perinatal service of the Long Beach Memorial Women's Hospital either primarily or as a perinatal and/or maternal transport with PROM and singleton pregnancies between 25 weeks and 30 weeks, 6 days of gestation were requested to consent to randomization. Gestational age was determined by menstrual history and prenatal examinations or if these were questionable, by ultrasound measurements of biparietal diameter, abdominal circumference, and femur length. Patients with heart disease, diabetes mellitus, and thyrotoxicosis were excluded.

Ruptre of membranes was documented by speculum examination that confirmed the pooling of fluid in the

From the Long Beach Memorial Women's Hospital, and the University of California, Irvine.

Supported by a grant from the Long Beach Memorial Medical Center Foundation.

Received for publication January 16, 1987; accepted April 28, 1987.

Reprint requests: Thomas J. Garite, M.D., Long Beach Memorial Women's Hospital, 2801 Atlantic Avenue, Long Beach, CA 90801.

posterior vaginal fornix; alkaline pH by Nitrazine paper; ferning; and direct visualization of leakage of fluid from the cervical canal.

Cultures for group B *Streptococcus* and gonorrhea were obtained at this time. No digital examinations were performed unless the patient was thought to be in active labor; although some patients had been examined digitally before maternal transport. Patients with equivocal or undocumented rupture of membranes were not included in the study. Patients in advanced active labor, as defined by regular contractions and clear visibility of the fetal presenting parts on speculum examination, were also excluded. Patients were then evaluated for fetal distress by continuous electronic fetal heart rate monitoring and for clinical chorioamnionitis. The clinical diagnosis of chorioamnionitis required the presence of a temperature elevation of ≥100.4° F and any one of the following: maternal tachycardia (heart rate >100 bpm); fetal tachycardia (heart rate >160 bpm); maternal leukocytosis (white blood cells >15,000/mm³); uterine tenderness; or foul-smelling amniotic fluid. Patients with irreversible fetal distress or clinical chorioamnionitis were delivered immediately and were not randomized.

Consenting patients were randomized to receive either expectant management only or expectant management plus intravenous administration of ritodrine at the onset of contractions. Patients in the expectant management group were delivered for spontaneous labor, clinical chorioamnionitis, or fetal distress. Patients in the tocolytic group were similarly managed except at the onset of contractions with a frequency of three or more per 20 minutes; tocolytic therapy with intravenously administered ritodrine was begun. Patients were started on 150 μg/min, which was increased by 50 μg every 10 minutes until one of the following was achieved: decrease in contraction frequency to <one contraction/10 min; unacceptable complications or side effects; or when a maximum of 350 μg/min was reached. The infusion was continued for at least 12 hours after contractions abated. The maximum dosage was allowed for no more than 24 hours in the event that contractions were continuing at this drug level. Patients whose labors were successfully stopped were switched to oral ritodrine at an initial dosage of 10 mg every 3 hours, but the dosage was adjusted frequently to that needed to keep contractions at a minimum while still keeping the maternal pulse under 110 bpm. The patient was treated a second time with intravenous ritodrine as necessary. Patients with labor but with clinical infection or fetal distress were not given ritodrine. Additionally, patients reaching the end of week 31 were no longer eligible for tocolysis. Those who had been started on intravenous or oral administration of ritodrine and were still receiving either were discontinued at that point. After 31 weeks of gestation, patients were managed expectantly only as in the first group. No corticosteroids were used in either group; patients who had received corticosteroids before transfer were excluded from randomization.

Table I. Prerandomization characteristics of study groups

	Tocolysis group	*Expectant group*	*p*
N	39	40	
Maternal age (mean)	26.4	28.9	NS
Parity			
0	19	15	NS
≥1	20	25	NS
Contractions on admission			
Regular	4	1	NS
Irregular	13	12	NS
None	22	27	NS
Gestational age on admission (wk)	27.9	27.3	NS
Duration of ROM on admission			
<12 hr	31	28	
12-24 hr	4	5	
24-48 hr	2	2	
>48 hr	2	5	NS
Underwent amniocentesis	9	3	NS
Digital cervical examinations before labor	2	5	NS
Temperature on admission			
<98.7° F	24	23	NS
98.7°-99.9° F	15	17	NS
GBS on cervical culture	1	3	NS
Baseline FHR on admission			
<140 bpm	13	9	
140-150 bpm	18	22	
>150 bpm	8	9	NS

ROM = Rupture of membranes; GBS = group B *Streptococcus;* FHR = fetal heart rate; NS = not significant.

Whenever possible, ultrasound-directed amniocentesis was performed for Gram stain and culture of amniotic fluid as soon as possible after admission. Patients with successful amniocentesis and Gram stain positive for bacteria were excluded from the study. However, patients did not require amniocentesis to be eligible. In general, patients not in labor were kept on continuous fetal heart rate monitoring for at least 24 hours and if stable were then transferred to the antepartum unit and kept in the hospital until delivery. On the antepartum unit they were monitored with frequent temperature readings and daily prolonged (1 hour) nonstress tests. Patients with abnormal or concerning nonstress tests were transferred back to labor and delivery for continuous fetal heart rate monitoring.

The route of delivery for both groups was determined by means of the same criteria. All breech presentations were delivered by cesarean section. When induction of labor was necessary or spontaneous labor

Table II. Time interval of PROM to delivery

	TG	*Received tocolysis*	*EG*	*p*
N	39	23	40	
Mean (days)	11.5	6.1	12.0	NS
>24 hr	35 (88%)	20 (87%)	36 (90%)	NS
>48 hr	30 (77%)	15 (65%)	30 (75%)	NS
>1 wk	12 (31%)	6 (26%)	13 (33%)	NS
Reached 32 wk	8 (21%)	3 (13%)	6 (15%)	NS

TG = Tocolytic group; EG = expectant group.

Table III. Reasons for delivery

Tocolysis group	
Labor/failed tocolysis	12
Infection (with or without labor)	14
Labor/reached 32 wk	8
Advanced labor	4
Labor/refused further tocolysis	1
Expectant group	
Labor (with or without infection)	32
Antepartum fetal distress	4
Induced because of infection	3
Significant maternal vaginal bleeding	1

occurred, cesarean section was performed for the usual obstetric indications. The presence of chorioamnionitis did not significantly alter indications for cesarean section or the duration of labor allowed. Prophylactic antibiotics were used on all patients delivered by cesarean section after cord clamping but in no patient who was delivered vaginally.

All maternal patient data were coded prospectively. Neonatal data were coded after neonatal discharge. The diagnosis of respiratory distress syndrome (RDS) was made only when clinical signs and chest x-ray films were confirmatory and the newborn infant required at least 24 hours of oxygen therapy.

Analysis of data was performed by means of χ^2 and two-tailed *t* tests.

Results

The study was conducted between January 1983 and September 1986. Seventy-nine patients were randomized; 39 to the tocolysis group and 40 to the expectant management group. Comparison of the prerandomization characteristics of both groups (Table I) would suggest that the two groups were well matched. In addition, there was no significant difference in the number of patients undergoing amniocentesis (nine of 39 in the tocolysis group vs 3 of 40 in the expectant group, $\chi^2 = 2.6$; not significant) or in the sex of the babies delivered (20 males/19 females in the tocolytic group vs 20 males/20 females in the expectant group) between the two groups.

Of the 39 patients randomized to receive tocolysis, 23 actually did receive ritodrine when contractions began. Of the 16 randomized to receive the drug but who did not, seven were ineligible because of clinical chorioamnionitis, five had gone beyond 31 weeks of gestation at the onset of labor, three had advanced cervical dilation at the time the drug was to be started, and one was infected and the fetus had no heart activity (stillborn).

The interval between the rupture of membranes and delivery in the two groups is compared in Table II. In addition, the subgroup of patients who actually received tocolytic therapy is separated for comparison. Both mean and intervallic data fail to show any prolongation of pregnancy with the addition of ritodrine to the more standard expectant management at the onset of labor. In addition, eight of 40 (20%) in the tocolytic group vs six of 40 (15%) in the expectant group (difference, not significant) reached 32 weeks of gestation, which in essence was the goal or point beyond which management in the two groups became similar. The causes and/or indications for delivery in both groups are listed in Table III.

Of the 23 patients who did receive ritodrine, 10 were delivered in <24 hours from the onset of therapy, six between 24 and 48 hours, one between 2 and 3 days, and six were delivered after the third day from the initiation of tocolytic therapy (4, 5, 6, 8, 11, and 23 days). There were no serious complications directly attributable to the ritodrine therapy in these patients, although one patient declined to be treated again after one full course when labor recurred because of side effects experienced with the first course.

Evaluation of maternal outcome in both groups does not reveal any striking dissimilarities. The cesarean section rate was similar between the two groups (18 of 39 [46%] in the tocolytic group vs 12 of 40 [30%] in the expectant group, $\chi^2 = 1.55$; not significant). The cesarean section rate was also similar in those patients who actually received ritodrine (eight of 23 [35%]). The incidence of chorioamnionitis was 14 of 39 (35%) in the tocolytic group vs seven of 40 (18%) in the expectant group; this difference was not statistically significantly different ($\chi^2 = 2.54$, $p = 0.12$). The incidence of cho-

Table IV. Neonatal outcome

	Tocolytic group	*Expectant group*	*p*
N	39	40	
Mean birth weight (gm)	1387	1340	NS
Gestational age at delivery (wk)	29.5	28.9	NS
RDS	20 (51%)	23 (58%)	NS
Sepsis	1 (3%)	2 (5%)	NS
IVH, grades 3 and 4	3 (8%)	2 (5%)	NS
Low Apgar scores (<7 at 5 min)	7 (18%)	2 (5%)	NS
Hospital stay (days)*			
Mean	47.5	57.0	NS†
>30	20 (60%)	26 (68%)	NS
>60	10 (30%)	12 (31%)	NS
Range	2-134	2-210	

RDS = Respiratory distress syndrome; IVH = intraventricular hemorrhage.
*Includes only babies discharged home alive (tocolytic group = 33, expectant group = 38).
†$t = 1.89$, $p = 0.08$.

rioamnionitis in those patients who actually received tocolysis was eight of 23 (30%). The occurrence of postpartum endomyometritis was also similar, with 10 of 39 (26%) in the tocolytic group and six of 40 (15%) in the expectant group but three of 23 (13%) in patients who received ritodrine. The incidence of prolonged postpartum stay (>3 days in vaginally delivered and >6 days for patients who had cesarean sections) was virtually identical between the two (three of 39 [8%] in the tocolytic group vs three of 40 [8%] in the expectant group).

Comparisons of neonatal outcome are found in Table IV. It can be seen that the birth weights, gestational ages at delivery, low frequencies of 5-minute Apgar scores, and incidences of significant neonatal complications are quite similar between the two groups. Although the mean hospital stays are numerically dissimilar between the two groups, this is not a normal curve, and the incidences of prolonged stays (>30 and >60 days) are quite similar. There were eight perinatal deaths among the 79 total patients in the study (101 of 1000). Two occurred in the expectant group (5%), including one stillbirth. There were six deaths in the tocolytic group (15%), also with one stillbirth. However, only two deaths (9%), both neonatal, occurred in the group that actually received tocolysis. The causes of death are listed in Table V.

Table V. Causes of perinatal deaths

Tocolytic group (6)	
Severe respiratory distress syndrome	4*
Trisomy 21/endocardial cushion defect	1
Sepsis—group B *Streptococcus* (stillbirth)	1
Expectant group (2)	
Necrotizing enterocolitis	1
Asphyxia/abruptio placentae (stillbirth)	1

*Possible sepsis (1); possible pulmonary hypoplasia (1).

Comment

Because prematurity is the leading cause of perinatal mortality in patients with PROM, and since recent studies[3, 4] have suggested increased maternal safety in those patients not delivered immediately after PROM, most authors[1] have recently recommended various management schemes that are chiefly aimed at prolonging pregnancy. The addition of tocolytic therapy to help achieve these goals in such patients is an obvious alternative.

In the past, tocolytic agents were said to be contraindicated in patients with PROM[2] because of presumed lack of efficacy and concern about aggravating infectious morbidity. Although there are indeed theoretical reasons to believe that both of these concerns may be valid, there have been few investigative data on which to corroborate or refute them.

Most of the current information refers to the relative effectiveness of tocolytic agents in patients with PROM compared with patients with premature labor with intact membranes. Caritis et al.[5] used terbutaline and were able to gain a week or more in only 14% of patients with ruptured membranes as opposed to 59% of patients with unruptured membranes. Elliott[6] evaluated magnesium sulfate as a tocolytic agent and showed that 78% of patients with intact membranes were still undelivered after 48 hours as opposed to only 5% whose membranes were ruptured. In a collaborative study Curet et al.[7] were able to show a reduction of over 50% in the rate of RDS in patients with premature labor who are given ritodrine, but in patients with PROM similarly treated there was no difference between treated patients and controls in the incidence of RDS. Christensen et al.[8] have published a small randomized

clinical trial in patients in preterm labor with PROM, comparing ritodrine therapy with placebo. The only difference shown was a prolongation of pregnancy beyond 24 hours in the tocolytic group, but beyond 48 hours no difference was shown. There was no improvement seen in outcome for any newborn. However, there was no apparent increase in the rate of infectious complications in either mothers or neonates.

In designing this randomized clinical study, the question that we asked was whether the planned addition of a tocolytic agent at the onset of labor was of any benefit in terms of prolonging pregnancy and/or improving neonatal outcome as compared with expectant management alone. Because it is probably true that tocolysis with PROM cannot be expected to be as effective as with preterm labor with intact membranes, we chose to study only those patients with very early gestational ages for whom any prolongation of pregnancy would be expected to result in the greatest benefit to the newborn. Goldenberg et al.[9] have suggested that between the gestational ages of 25 and 30 weeks, improvements in survival of 0.9% to 3% per day and 6% to 21% per week could be expected.

The results of this clinical trial do not suggest that there is any benefit to adding tocolysis to expectant management either in terms of prolonging the interval between PROM and delivery or in reducing morbidity from prematurity or perinatal mortality. As seen in Table II, there is a striking similarity in the time intervals between PROM and delivery for the two groups both in terms of mean days gained and in the intervallic data. In terms of the overall goal, that is, to reach 32 weeks of gestation, since that was the time when management became similar in the two groups, no difference was seen.

With respect to neonatal outcome, virtually all parameters evaluated were similar between the two groups. This is true for birth weights and gestational ages at delivery and for morbidity of prematurity and the duration of neonatal hospital stay. It would be expected that any neonatal benefit from prolonging pregnancy with tocolysis in these patients would be shown in these outcome parameters, and none were seen.

It is tempting to try to compare the 23 patients who actually received ritodrine with a similar group of patients from the expectant management group. However, one cannot validly extract from the expectant group which patients should be compared and at what point in time the onset of labor for these patients should be defined, since no attempt was made prospectively to identify them. Valid comparisons should not be made between this group that actually received tocolysis and the overall expectant group, since the latter includes patients who went beyond 32 weeks of gestation, infected patients, and those who were in advanced labor when their labors were first discovered. Therefore one cannot conclude from this study that tocolysis in laboring patients with PROM shows no apparent benefit over no tocolysis, as was suggested in the study by Christensen et al.[8] Rather, what was shown was that the addition of planned tocolysis to expectant management did not demonstrate any apparent benefit over expectant management alone.

One additional point that this study illustrates is not only lack of apparent efficacy but limited applicability, since only 23 of 39 (59%) patients randomized to receive ritodrine actually did so. Most frequently this was because of infection or advanced labor at the time that diagnosis of labor was made. The impact of this consideration is important, since even if limited efficacy could be shown with tocolysis in patients with PROM in larger studies, the fact would remain that the overall impact would be limited by lack of complete applicability. This problem has also been identified in premature labor in general.[2]

Although this study does not demonstrate any apparent benefit from tocolysis, it does not suggest any deleterious effect from such treatment in patients with PROM. There was no increase in the incidence of any infectious complication. Though there is a numerical trend toward more chorioamnionitis in the tocolytic group, this trend was not limited to those patients who actually received tocolysis, for whom the incidence was actually less (seven of 23 [30%]) than in the overall tocolytic group (14 of 39 [35%]). However, it is possible that a larger study might demonstrate such deleterious effects. Additionally, there were no other apparent harmful maternal or neonatal effects.

Perhaps the reason that no benefit was shown with the addition of tocolytic therapy in this study is that once labor has been estabished in patients with PROM it is already too late. Perhaps earlier detection of contractions and earlier institution of therapy might improve efficacy. One choice might be frequent contraction monitoring with a device such as that described by Katz et al.,[10] the "ambulatory tokodynamometer." Alternatively or additionally, prophylactic oral agents might be of benefit as shown by Levy and Warsof,[11] who showed in a prospective randomized study that oral ritodrine given to patients not in labor with PROM substantially prolonged pregnancy over control patients; however, although babies were slightly larger in the treatment group, no other improvement in neonatal outcome was shown.

Although current studies may show that short-term gains such as 24 or 48 hours may be accomplished with intravenous therapy once labor is started, there is a question of whether there are any benefits to be derived

from such short delays. The benefit of corticosteroids given to the mother to reduce neonatal RDS is at best questionable in patients with PROM and may increase the risk of infection.[1] Delaying delivery 24 to 48 hours alone in the presence of PROM has been suggested to lower the chances of RDS, but again these effects are controversial.[12]

In conclusion, although this study is limited by relatively small numbers, it was conducted in those gestational ages during which prolongation of pregnancy might have been expected to produce the greatest benefit in neonatal outcome. Nevertheless, no benefit could be demonstrated with the addition of a proved effective tocolytic agent after the onset of labor over expectant management alone in the patient with PROM in the very premature gestational period. Some form of expectant management with careful attention to avoiding complications from infection and asphyxia remains the management of choice for providing the best outcome in patients with PROM. It appears that tocolysis at the onset of labor will do little to improve on this form of management although further studies certainly should be done to confirm or refute this conclusion.

REFERENCES

1. Garite TJ. Premature rupture of membranes. Curr Probl Obstet Gynecol 1984;7:1.
2. Zlatnik FJ. The applicability of labor inhibition to the problem of prematurity. AM J OBSTET GYNECOL 1972; 113:704.
3. Koh KS, Chan FH, Manfared AH, et al. The changing maternal and perinatal outcome in chorioamnionitis. Obstet Gynecol 1979;53:730.
4. Garite TJ, Freeman RK. Chorioamnionitis in the preterm gestation. Obstet Gynecol 1982;54:539.
5. Caritis SN, Toig G, Heddinger LA, et al. A double-blind study comparing ritodrine and terbutaline in the treatment of preterm labor. AM J OBSTET GYNECOL 1984; 150:7.
6. Elliott JP. Magnesium sulfate as a tocolytic agent. AM J OBSTET GYNECOL 1983;147:277.
7. Curet LB, Rao AV, Zachman RD, et al. Association between ruptured membranes, tocolytic therapy, and respiratory distress syndrome. AM J OBSTET GYNECOL 1984;148:263.
8. Christensen KK, Ingemarsson I, Leideman T, et al. Effect of ritodrine on labor after premature rupture of membranes. Obstet Gynecol 1980;55:187.
9. Goldenberg RL, Nelson KG, Davis RO, et al. Delay in delivery: influence on gestational age and the duration of delay on perinatal outcome. Obstet Gynecol 1984;64:480.
10. Katz M, Newman RB, Gill PJ. Assessment of uterine activity in ambulatory patients at risk of preterm labor and delivery. AM J OBSTET GYNECOL 1986;154:44.
11. Levy DL, Warsof SL. Oral ritodrine and preterm premature rupture of membranes. Obstet Gynecol 1985; 66:621.
12. Mead PB. Management of the patient with premature rupture of the membranes. Clin Perinatol 1980;7:243.

Detection of *Chlamydia trachomatis* cervical infection: A comparison of Papanicolaou and immunofluorescent staining with cell culture

Thomas C. Quinn, M.D., Prabodh K. Gupta, M.D., Ronald T. Burkman, M.D., Elizabeth W. Kappus, B.S., Marguerite Barbacci, B.S.N., M.P.H., and Michael R. Spence, M.D., M.P.H.

Bethesda, Maryland

We compared tissue culture with Papanicolaou-stained cervical smears, endocervical cytologic smears stained with immunofluorescent monoclonal antibody, and a direct immunofluorescent stain of cervical specimens (MicroTrak) for detection of *Chlamydia trachomatis* in cervical specimens. Fifty-one (21%) of 245 women had positive cultures for *C. trachomatis*, 14 (27%) of whom had clinical evidence of cervicitis. With the criteria of intracytoplasmic coccoid inclusion bodies within metaplastic cells, 45 (34%) of 130 Papanicolaou smears were read as suggestive for *C. trachomatis*. Seventeen of the 45 positive Papanicolaou smears were positive on culture and 28 were negative (sensitivity 54%, specificity 71%). In contrast, 48 of 51 women with positive cultures and one woman with a negative culture had positive immunofluorescent-stained cytologic smears (sensitivity 94%, specificity 99%, with positive predictive value of 98%). Similarly, 47 of 51 women with positive cultures also had positive results with MicroTrak direct immunofluorescent stain, with only one positive specimen in 196 women with negative cultures (sensitivity 92%, specificity 99%, with positive predictive value of 98%). This study demonstrates that immunofluorescent staining of cervical specimens or of cytologic smears is a more sensitive and specific method than routine Papanicolaou smear for detection of chlamydia infection in a high-prevalence population. (Am J Obstet Gynecol 1987;157:394-9.)

Key words: *Chlamydia trachomatis*, Papanicolaou smear, cervicitis, immunofluorescent staining

Chlamydia trachomatis is presently the most frequent sexually transmitted bacterial pathogen in the United States. It is associated with a wide variety of genital and ocular infections including mucopurulent cervicitis, endometritis, salpingitis, urethritis, epididymitis, proctitis, neonatal and adult inclusion conjunctivitis, trachoma, and pneumonia of newborn infants and immunosuppressed adults.[1] Despite the wide variety of clinical syndromes and associated morbidity, screening for chlamydia infections is still not widely performed by physicians in the United States. The standard method for screening for chlamydia is in vitro tissue culture of specimens. Cultures, however, are not widely available, are costly, demand strict transport requirements, and take approximately 5 days before results are made available to the physician. More recently, several new diagnostic assays for chlamydia were licensed in the United States. These include a direct immunofluorescent monoclonal antibody stain and enzyme-linked immunoabsorbent assays that detect chlamydial antigen in the cervical and urethral secretions. Previous studies have demonstrated that these assays are rapid, inexpensive, and relatively sensitive and specific compared with culture.[2-4]

An alternative method of detection of chlamydial infection has been by cytologic identification on routine Papanicolaou smears. Gupta et al.[5] suggested that chlamydia could be diagnosed on routine Papanicolaou smears by the identification of intracytoplasmic coccoid inclusion bodies in enlarged metaplastic cells. More recently, Shafer et al.[6, 7] demonstrated that increased numbers of histiocytes, polymorphonuclear leukocytes, and transformed lymphocytes were significantly associated with chlamydia infection. Using these criteria, Kiviat et al.[8] suggested a two-step method by which cytologic Papanicolaou smears suspicious for chlamydia could be used for the selection of patients for confirmatory testing.[9]

Although there has been controversy regarding the reliability of some of these criteria for the diagnosis of chlamydia,[7, 10] certainly a sensitive and specific test that includes use of routine cytologic smears for the detec-

From the National Institute of Allergy and Infectious Disease, National Institute of Health, and the Departments of Medicine, Pathology, and Obstetrics and Gynecology, The Johns Hopkins University School of Medicine.

Received for publication October 24, 1986; revised February 9, 1987; accepted March 2, 1987.

Reprint requests: Thomas C. Quinn, M.D., Blalock 1111, The Johns Hopkins Hospital, Baltimore, MD 21205.

tion of chlamydia would be advantageous for screening of women, since approximately 25 million women undergo cytologic screening annually in the United States. We therefore undertook a study to compare several of these diagnostic methods for the detection of cervical chlamydial infection.

Material and methods

Study population. Patients were prospectively enrolled from three clinics at The Johns Hopkins Hospital and the Baltimore City Sexually Transmitted Diseases (STD) Clinic. The study group consisted of 204 (85%) pregnant women admitted for an abortion procedure, 22 (9%) evaluated at the STD clinic, and 19 (6%) attending the obstetrics-gynecology clinic for routine examination. Patients who had received antibiotics in the month prior to enrollment were excluded from the study.

Patient examination. Information concerning demography, menstrual history, contraceptive methods, sexual history, and history of sexually transmitted diseases was obtained from all women. All patients underwent a physical and pelvic examination. Information regarding the type of vaginal discharge, the color of cervical secretions as viewed on a cotton-tipped swab,[11] the presence or absence of cervical friability and bleeding, the presence of cervical ectopy, and the presence of erythema or edema in the area of ectopy was recorded. Patients were diagnosed as having cervicitis if cervical friability, hypertrophic ectopy, and a mucopurulent cervical discharge were present.[12]

Collection of specimens. Specimens obtained from each patient included a cervical smear for cytologic examination (Papanicolaou smear), a cervical smear for immunofluorescent staining, an endocervical swab for isolation of *Neisseria gonorrhoeae*, and an endocervical swab for direct immunofluorescent staining (MicroTrak, Syva Corp., Palo Alto, California) and tissue culture isolation of *C. trachomatis*. Specimens for each test were collected according to computer-generated random order.

Cultures for *N. gonorrhoeae* were obtained by inserting a wooden shaft, cotton-tipped swab into the endocervical canal, rotating for 15 seconds, and immediately inoculating it onto Thayer-Martin media, followed by incubation and identification by standard methods.[13]

Specimens for isolation and detection of *C. trachomatis* by tissue culture assay and direct immunofluorescent staining were obtained with a Dacron polyester fiber–tipped plastic swab (American Scientific Products, McGaw Park, Illinois), which was then rolled onto a circular area 8 mm in diameter of a microscope slide (MicroTrak) and placed in 1.5 ml of 0.2 mmol/L sucrose-phosphate–buffered transport media. The slides were air-dried and fixed in 2 ml of water-free acetone. Culture samples and slides were transported to the laboratory and frozen at −70° C for 24 hours.

Laboratory procedures. Tissue culture isolation of *C. trachomatis* was carried out in 96-well microtiter plates on McCoy cell monolayers as previously described.[14] After incubation for 72 hours at 35° C, two wells were stained with 30 μl of fluorescein-conjugated monoclonal antibody (Syva Corp.). The remaining two wells were disrupted and passaged onto fresh McCoy cell monolayers for another 72 hours. Culture monolayers were examined for characteristic inclusions at ×100 magnification on a Zeiss fluorescence microscope. The presence of one inclusion was considered positive for *C. trachomatis*.

MicroTrak slides were stained with 30 μl of fluorescein-conjugated monoclonal antibody against *C. trachomatis* (Syva Corp.). After an incubation of 15 minutes at room temperature, the slides were rinsed in distilled water, air dried, and scanned at ×630 magnification on a Zeiss fluorescent microscope. Typical elementary bodies appear as well-defined, sharply edged disks of apple green fluorescence.[2] The presence of five or more elementary bodies was the criterion used for positive identification.

Endocervical scrapings for immunofluorescent detection of *C. trachomatis* were removed from fixative, rinsed in phosphate-buffered saline, and stained with 100 μl of fluorescein-conjugated monoclonal antibody to *C. trachomatis* (Syva Corp.). A coverslip was applied to each slide to ensure even coverage of the antibody and the slides were incubated for 30 minutes at room temperature in a humidified atmosphere. The slides were rinsed in distilled water, allowed to air dry, and covered with a 22 × 50 mm coverslip and mounting fluid. The entire slide was then scanned at ×630 magnification on a Zeiss fluorescent microscope. The presence of ≥10 elementary bodies per slide was the criterion used for positive identification of *C. trachomatis* on cytologic smears because of the larger surface area of the slide to be examined in contrast to the 8 mm diameter area of a MicroTrak slide.

Cytologic methods. A duplicate cervicovaginal smear was sent to the cytopathology laboratory and stained with a modified Papanicolaou stain. All cytologic slide evaluations were conducted by one person (P. K. G.) and a special form was used for each case. The presence or absence of endocervical cells, metaplastic cells, and blood was noted. The presence of each type of inflammatory cell (polymorphonuclear leukocytes, lymphocytes, plasma cells, and histiocytes) was quantified and the epithelial cytologic findings were described in detail. Assessment for *C. trachomatis* infection was first performed with previously published criteria,[5] including metaplastic cell morphologic features, the finding

Table I. Demographic and clinical signs of infection in a population in relationship to chlamydia culture results

Patient characteristic	C. trachomatis *culture result*					
	Positive		*Negative*		*Total*	
	n	%	*n*	%	*n*	%
No. of patients	51	21	194	79	245	
Age (yr)						
Mean	18.6 ($p < 0.0001$)*		22.4		21.8	
Range	13-30		12-39		12-39	
Ethnic origin						
Black	45	88	148	76	193	79
White	5	10	46	24	51	21
Other	1	2	0	0	1	.01
Marital status						
Unmarried	49	96	177	91	226	92
Married	2	4	17	9	19	8
Evidence of cervicitis‡	14	27 ($p < 0.007$)†	22	11	36	15
Cervical friability	36	71 ($p < 0.01$)†	98	51	134	55
Cervical ectopy	31	61 ($p < 0.02$)†	76	39	107	44
Mucopurulent cervical discharge	20	31 ($p < 0.002$)†	34	18	54	22

*Student's *t* test.

†χ^2 analysis.

‡Cervicitis was defined as the presence of all three criteria—cervical friability, cervical ectopy, and a mucopurulent cervical discharge.

of intracytoplasmic coccoid inclusion bodies in metaplastic cells, and multinucleation and intracytoplasmic vacuolation of these cells. Cytologic slides were then reanalyzed for chlamydia according to criteria recently described by Kiviat et al.[8] Chlamydia infection was suspected by these criteria if there were ≥30 histiocytes in at least three fields, if transformed lymphocytes were present, or if there were increased numbers of leukocytes and plasma cells.

MicroTrak smears, immunofluorescent cervical smears, and cervical Papanicolaou smears were read independently by three investigators that were blinded to the other laboratory results or clinical information.

Statistical analyses. Differences in proportions were tested for significance by χ^2 analysis and means were compared by use of Student's *t* test. Univariate and multiple logistic regression analyses were used to determine associations between clinical signs of infection, epithelial cell changes, and presence or absence of cervical pathogens.

Results

Isolation of *C. trachomatis* by tissue culture assay. Fifty-one (21%) of the 245 women enrolled in the study had positive cervical cultures for *C. trachomatis*. Women infected with chlamydia were significantly younger, with a mean age of 18.6 years, than chlamydia-negative women, who had a mean age of 22.4 years ($p < 0.0001$; Table I). There were no significant differences noted in racial or marital status, which were predominantly black (79%) and unmarried (92%), respectively (Table I).

Fourteen (27%) of the 51 chlamydia-positive women had cervicitis on examination compared with 22 (11%) of 194 women with negative cultures ($p < 0.007$; Table I). In univariate analysis of the clinical variables of cervicitis, the presence of a mucopurulent cervical discharge ($p < 0.002$), cervical ectopy ($p < 0.02$), and cervical friability ($p < 0.01$) was associated with chlamydial infection (Table I).

Evaluation of cytologic screening by Papanicolaou smears for *C. trachomatis* infection. The reliability of Papanicolaou smear diagnosis of chlamydial infections compared with in vitro culture was examined by means of standard cytologic criteria.[7] Of the 245 specimens obtained, 115 were classified as inadequate because of insufficient endocervical components (95 specimens), excessive inflammation with cellular obscuring (23), excessive cytolysis (4), and heavy fresh or old blood (15). Of the remaining 130 specimens, 45 (35%) were interpreted as positive for *C. trachomatis*. Of 28 women with positive cultures and evaluable cytologic smears, 15 had Papanicolaou smears interpreted as positive for *C. trachomatis* and 13 had smears interpreted as negative (Table II). Of the 102 women with negative cultures and evaluable cytologic smears, 30 had Papanicolaou smears interpreted as positive and 72 had smears interpreted as negative (Table II). Thus, in this relatively high-prevalence population for *C. trachomatis* infection, an evaluable cytologic Papanicolaou smear had a sen-

Table II. Comparison of culture for *C. trachomatis* with evaluable cytologic Papanicolaou smears according to standard and modified criteria

Test	C. trachomatis *culture result*		Total
	Positive	*Negative*	
Standard cytology–Papanicolaou smear*			
Positive (n)	15	30	45
Negative (n)	13	72	85
Sensitivity (%)			54
Specificity (%)			71
Positive predictive value (%)			33
Negative predictive value (%)			85
Modified cytology–Papanicolaou smear†			
Positive (n)	16	30	46
Negative (n)	35	164	199
Sensitivity (%)			31
Specificity (%)			84
Positive predictive value (%)			35
Negative predictive value (%)			82

*Only 130 slides were evaluable by standard cytologic criteria for *C. trachomatis* screening.

†The modified cytologic criteria incorporate the finding of inflammatory cells for detection of *C. trachomatis*. A total of 245 slides were evaluable by this method.

sitivity of 54%, a specificity of 71%, a positive predictive value of 33%, and a negative predictive value of 85% when compared with culture.

In a reanalysis of cytologic diagnosis for *C. trachomatis*, all Papanicolaou smears were screened according to criteria described by Kiviat et al.[8] Slides with increased histiocytes, leukocytes, plasma cells, and/or transformed lymphocytes were interpreted as positive for *C. trachomatis*. Of 51 women with positive cultures, 16 had Papanicolaou smears interpreted as positive for chlamydia. Of the 194 women with negative cultures, 164 had negative Papanicolaou smears for chlamydia. Thus, with these modified criteria of Kiviat et al.,[8] the Papanicolaou smear had a sensitivity of 31%, a specificity of 84%, a positive predictive value of 35%, and a negative predictive value of 82%.

Univariate analysis of Papanicolaou smear findings and detection of *C. trachomatis* by culture showed that only the presence of polymorphonuclear leukocytes significantly correlated with a positive chlamydia culture (Table III). The presence of metaplastic cells, histiocytes, plasma cells, or lymphocytes did not correlate significantly with culture results. In addition, the presence of *Trichomonas vaginalis* in Papanicolaou smears of 12 (43%) of 28 patients with positive cultures versus 17 (17%) of 102 patients with negative cultures ($p < 0.007$) correlated with positive culture results for *C. trachomatis*.

Evaluation of direct immunofluorescence, staining of cytologic smears, and cervical specimens (MicroTrak). Cytologic smears were analyzed for *C. trachomatis* infection after staining with fluorescein-conjugated monoclonal antibody against *C. trachomatis*. All 245 duplicate cytologic smears were evaluated, and

Table III. Correlation of cytologic findings on Papanicolaou smears with *C. trachomatis* culture results

Cytologic finding	χ^2	*p Value*
Squamous metaplastic cells		
Cytovacuoles	0.08	0.77
Cytogranules	0.34	0.56
Cytoinclusions	0.37	0.54
Inflammatory cells		
Plasma cells (>0)	0.24	0.62
Histiocytes (≥1+)	0.35	0.56
Lymphocytes (>0)	0.96	0.33
Polymorphonuclear leukocytes (≥3+)	5.07	0.02

no slides were interpreted as inadequate. Forty-eight (94%) of 51 patients with positive cultures had positive immunofluorescent-cytologic smears and 193 (99%) of 194 women with negative cultures had negative smears (Table IV). The sensitivity and specificity of immunofluorescent-cytologic smears were 94% and 99%, respectively. The positive and negative predictive values were each 98%.

Staining with monoclonal antibody occasionally identified chlamydia inclusions on cytologic smears. Of the 48 Papanicolaou smears that were read as positive for chlamydia only nine had inclusions present on immunofluorescent-stained cytologic smears. Thus 36 culture-negative and immunofluorescent-negative smears were falsely interpreted as showing chlamydia inclusions on Papanicolaou-stained smears.

MicroTrak direct immunofluorescent staining of endocervical smears was also compared with chlamydia cultures. Forty-seven (92%) of 51 women with positive cultures had positive smears and 193 of 194 women

Table IV. Comparison of direct immunofluorescent staining of cytologic smears and endocervical specimens by MicroTrak

Staining	C. trachomatis *culture result*		*Total*
	Positive	*Negative*	
Immunofluorescent stain of cytologic smears			
Positive (n)	48	1	49
Negative (n)	3	193	196
Sensitivity (%)			94
Specificity (%)			99
Positive predictive value (%)			98
Negative predictive value (%)			98
Immunofluorescent stain of cervical specimens (MicroTrak)			
Positive (n)	47	1	48
Negative (n)	4	193	197
Sensitivity (%)			92
Specificity (%)			99
Positive predictive value (%)			98
Negative predictive value (%)			98

with negative cultures had negative smears (Table IV). Thus, in this population, the MicroTrak direct immunofluorescent smear had a sensitivity and a specificity of 92% and 99%, with positive and negative predictive values of 98% and 98%, respectively.

Comment

This study compared four different methods currently recommended for screening of *C. trachomatis* infection in women: culture, Papanicolaou smear, direct immunofluorescent staining of cytologic smears, and direct immunofluorescent staining of a cervical smear (MicroTrak). Detection of chlamydia has become increasingly important because of its high prevalence in our population (5 to 10 million infected people), its high frequency of asymptomatic infections, and its associated morbidity. Chlamydial infections during pregnancy are a significant problem since they have been associated with prematurity, increased fetal wastage, postpartum and postabortal endometritis, and neonatal infections, such as conjunctivitis, pneumonia, and possibly otitis media.[11] However, screening of *C. trachomatis* in pregnant women is rarely performed. We therefore undertook this comparative study of diagnostic assays predominantly in women who were in the first trimester of pregnancy and some nonpregnant women from other clinics.

The population sampled had a relatively high infection rate of 21%, not uncommon for young, sexually active, inner-city black women.[5] Although young age and evidence of cervicitis correlated with chlamydia infection (Table I), the majority of chlamydia-infected women were asymptomatic. While the presence of a mucopurulent cervical discharge in a young, sexually active woman was highly suggestive of chlamydia infection,[12] its absence did not rule out the diagnosis.

The use of the Papanicolaou smear has been recommended for use in chlamydia screening for the last decade, but the reliability of this test for the detection of chlamydia has been controversial. Naib[15] originally described chlamydia-induced changes noted in Papanicolaou-stained smears of newborn infants with neonatal conjunctivitis and from their mothers. Gupta et al.[5] further described the cytologic findings of chlamydia cervicitis. Multinucleated, metaplastic cells with intracytoplasmic vacuoles or coccobacillary bodies on Papanicolaou smears became the standard criteria for diagnosis of chlamydia cervical infection. However, several subsequent studies demonstrated that this method was relatively insensitive for the detection of chlamydia.[7, 10, 16]

In this study, nearly half of the Papanicolaou smears could not be evaluated because of relatively strict criteria. For those that could be evaluated, the Papanicolaou smear had a sensitivity of 54%, a specificity of 71%, and positive and negative predictive values of 33% and 85%, respectively. Although chlamydia inclusions were suspected in 45 specimens, only 15 were culture positive, of which only nine had inclusions demonstrable by immunofluorescent staining. Thus cytologic diagnosis of chlamydia infection on Papanicolaou smear in predominantly pregnant women was insensitive.

More recently, several studies have demonstrated that nonspecific inflammatory cellular atypias were significantly associated with *C. trachomatis* infection.[6-8] In a study of 148 sexually active adolescents, Shafer et al.[7] found that 23 (15.5%) were infected with *C. trachomatis*. In multiple logistic regression analysis only inflammatory changes and lymphocytes correlated with chlamydia infection. In a subsequent study of 265 women with an incidence of 29%, Kiviat et al.[8] found that chlamydia infection was associated with the presence of

metaplastic cells, transformed lymphocytes, and increased histiocytes. These criteria for the detection of chlamydia had a sensitivity of 95%, a specificity of 75%, and a positive predictive value of 50%. Because of the high sensitivity of these criteria they suggested a two-step approach by which patients with a first Papanicolaou smear that was suspicious for *C. trachomatis* infection would have a second cytologic smear stained with monoclonal antibody to *C. trachomatis*. This two-step approach resulted in a sensitivity of 76%, a specificity of 100%, and a positive predictive value of 100%.[9]

By adopting the cytologic criteria of Kiviat et al.,[8] we found a relatively low sensitivity rate of 31%, a specificity rate of 84%, a positive predictive value of 35%, and a negative predictive value of 82% for Papanicolaou smear diagnosis. By univariate and multivariate logistic regression, we found that only the presence of increased polymorphonuclear leukocytes correlated with chlamydia isolation. Differences in patient populations may partially explain these differences since Kiviat et al.[8] excluded pregnant women and had a greater number of women with cervicitis. It may be that ectopy of pregnancy may alter the cytologic diagnosis of chlamydia, and further studies are warranted in nonpregnant women.

Our data support the finding that immunofluorescent staining of cytologic smears or of cervical specimens (MicroTrak) is highly effective in screening for *C. trachomatis*. Both tests have very high sensitivities and specificities when compared with culture (Table IV). Indeed in one case immunofluorescent staining may have detected a true positive specimen that was negative by culture. Positive and negative predictive values in high-prevalence populations have been 98% for both tests, but subsequent studies will have to determine what these values are for low-prevalence populations where the level of detectable antigen may be lower.

Routine immunofluorescent staining of cytologic smears should not be recommended, however, since the reading of these smears is much more labor intensive than immunofluorescent staining of cervical smears as in the MicroTrak assay. In this latter test, microscopic examination is limited to an area of the slide 8 mm in diameter, whereas in cytologic smears a greater area of the slide must be examined. Deficiencies ascribed to these tests include the dependency on technician proficiency in discriminating elementary bodies of chlamydia from artifacts and the potential for detecting antigen in successfully treated patients. Further studies are warranted for the evaluation of these tests in different patient populations.

In summary, our data suggest that routine screening of all high-risk individuals for chlamydia infection should be performed, particularly since the majority of our infected patients were asymptomatic, and that immunofluorescent staining of cervical smears (MicroTrak) and immunofluorescent staining of cytologic smears are sensitive and specific assays for the detection of *C. trachomatis*.

We thank Dr. Barbara Judson for providing monoclonal antibody and for computer analysis of data. We thank Rhonda Reese for preparation of the manuscript.

REFERENCES

1. Schacter J. Chlamydial infections. N Engl J Med 1978; 298:428-35, 490-5, 540-9.
2. Stamm WE, Harrison HR, Alexander ER, Cles LD, Spence MR, Quinn TC. Diagnosis of *Chlamydia trachomatis* infections by direct immunofluorescence staining of genital secretions—a multicenter trial. Ann Intern Med 1984;101:638-41.
3. Quinn TC, Warfield P, Kappus E, Barbacci M, Spence M. Screening for *Chlamydia trachomatis* infection in an inner-city population: a comparison of diagnostic methods. J Infect Dis 1985;152:419-22.
4. Chernesky MA, Mahony JB, Castriciano S, et al. Detection of *Chlamydia trachomatis* antigens by enzyme immunoassay and immunofluorescence in genital specimens from symptomatic and asymptomatic women. J Infect Dis 1986;154:141-8.
5. Gupta PK, Lee EF, Erozan YS, Frost JK, Geddes ST, Donovan PA. Cytologic investigations in Chlamydia infection. Acta Cytol 1979;23:315-20.
6. Shafer MA, Beck A, Blain B, et al. *Chlamydia trachomatis:* important relationships to race, contraception, lower genital tract infection, and Papanicolaou smear. J Pediatr 1984;104:141-6.
7. Shafer MA, Chew KL, Kromhout LK, et al. Chlamydial endocervical infections and cytologic findings in sexually active female adolescents. Am J Obstet Gynecol 1985; 151:765-71.
8. Kiviat NB, Paavonen JA, Brockway J, et al. Cytologic manifestations of cervical and vaginal infections. I. Epithelial and inflammatory cellular changes. JAMA 1985;253: 989-96.
9. Kiviat NB, Peterson M, Kinney-Thomas E, Tam M, Stamm WE, Holmes KK. Cytologic manifestations of cervical and vaginal infections. II. Confirmation of *Chlamydia trachomatis* infection by direct immunofluorescence using monoclonal antibodies. JAMA 1985;253:997-1000.
10. Forster GE, Cookey I, Munday PE, et al. Investigation into the value of Papanicolaou stained cervical smears for the diagnosis of chlamydial cervical infection. J Clin Pathol 1985;38:399-402.
11. Alexander ER, Harrison HR. Role of *Chlamydia trachomatis* in perinatal infection. Rev Infect Dis 1983;5:713.
12. Brunham RC, Paavonen J, Stevens CE, et al. Mucopurulent cervicitis—the ignored counterpart in women of urethritis in men. N Engl J Med 1984;311:1-5.
13. Martin JE, Armstrong JH, Smith PB. New system for cultivation of *Neisseria gonorrhoeae*. Appl Microbiol 1974;27:802-5.
14. Yoder BL, Stamm WE, Koester CM, Alexander ER. Microtest procedure for isolation of *Chlamydia trachomatis*. J Clin Microbiol 1981;13:1036-9.
15. Naib ZM. Cytology of TRIC agent infection of the eye of newborn infants and their mothers' genital tracts. Acta Cytol 1970;14:390.
16. Dorman SA, Danos LM, Wilson DJ, et al. Detection of Chlamydial cervicitis by Papanicolaou stained smears and culture. Am J Clin Pathol 1983;79:421-5.

Genetic heterogeneity of prematurity and intrauterine growth retardation: Clues from the Old Order Amish

Muin J. Khoury, M.D., Ph.D., and Bernice H. Cohen, Ph.D., M.P.H.
Baltimore, Maryland

We studied differences in the role of genetic factors in prematurity and intrauterine growth retardation with the use of data on 312 Amish singleton live children ascertained from Amish records in Lancaster county, Pennsylvania, between 1969 and 1980. Birth and death certificates were obtained on all children, and inbreeding coefficients of child, mother, and father were computed by use of the path method of tracing common ancestors in a unique genealogic registry of Amish ancestors dating back to the 1700s. Multivariate analysis with linear and log linear models showed that a lower mean gestational age and a higher risk of prematurity (<37 weeks) and borderline maturity (37 to 38 weeks) were significantly associated with increased maternal inbreeding but not child or paternal inbreeding. On the other hand, a higher risk of intrauterine growth retardation (less than the tenth percentile in birth weight for gestational age) and mild intrauterine growth delay (tenth to twenty-fifth percentile) were associated with increased child inbreeding but not maternal or paternal inbreeding. The analysis suggests the presence of genetic heterogeneity in the etiology of prematurity and intrauterine growth retardation; while prematurity is mostly related to the maternal genotype, intrauterine growth retardation is related to the fetal genotype. The study reemphasizes the need for separating low birth weight into prematurity and intrauterine growth retardation in genetic and epidemiologic studies. (Am J Obstet Gynecol 1987;157:400-10.)

Key words: Genetics, inbreeding, intrauterine growth retardation, Old Order Amish, prematurity

Low birth weight (<2500 gm) has long been recognized as an important predictor of infant mortality and childhood morbidity[1] and has been the focus of many epidemiologic studies[2] that have attempted to characterize its risk profiles in order to devise prevention strategies.[3] However, studies of the causes of low birth weight have infrequently examined the two components of low birth weight: prematurity (gestational age <37 weeks) and intrauterine growth retardation (IUGR, low birth weight for gestational age). Although the two entities have different morbidity and mortality patterns as well as different pathophysiologic and epidemiologic characteristics,[4, 5] only recently have observational and intervention studies begun to measure differences in risk factor associations between prematurity and IUGR.[5] Among these risk profiles, however, differences in genetic factors have rarely been explored. Although birth weight is known to be correlated among siblings,[6] only a few studies have examined whether such relationships are due to correlations in length of gestation or intrauterine growth or both.[7] This may be partly due to the lack of uniform definition of IUGR[8] as well as to the high degree of correlation between IUGR and prematurity.[9]

In this study, we use data from a unique population, the Old Order Amish, to describe the differential impact of child and parental inbreeding on the length of gestation and intrauterine growth. Our analysis suggests the existence of autosomal recessive genes affecting prematurity and IUGR. Moreover, the study suggests that, while prematurity is associated primarily with the maternal genotype, IUGR is associated with the fetal genotype.

Methods

The study population. The Old Order Amish is a religious isolate that has attracted the interest of researchers in many fields including sociology, genetics, epidemiology, and demography.[10] This interest stems from the unique characteristics of the Amish that facilitate inferences concerning the role of genetic factors in disease. Such characteristics include their reproductive isolation, close inbreeding, farming way of life, homogeneous sociocultural patterns, high standards of living, and large family size.[10] In addition, the Amish have kept extensive family and genealogic records dating back to the time of pioneer migrants in the 1700s.[11]

This study is part of a case-control investigation of the impact of inbreeding on prereproductive mortality

From the Department of Epidemiology, The Johns Hopkins University School of Hygiene and Public Health.
Received for publication September 30, 1986; revised April 6, 1987; accepted April 24, 1987.
Reprint requests: Dr. B. H. Cohen, Department of Epidemiology, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205.

in the Old Order Amish of Lancaster County, Pennsylvania.[12] Cases included all offspring who died at less than 20 years of age between 1969 and 1980 in the Lancaster County community. These were ascertained via multiple sources[10] including: (1) the Diary, which is an Amish monthly publication that publishes lists of births, deaths, and marriages among the Old Order Amish; (2) a 1980 Amish population directory, which is a complete population and family census of Lancaster County Amish; (3) death certificates obtained from the Pennsylvania State Health Department. On the other hand, controls included live children randomly selected from an annual listing of births published in the Diary as well as children listed in the 1980 directory. Controls were frequency matched to cases by year of birth and were alive as of December 31, 1980. In this analysis, only live-born singleton cases and controls were used and three groups were created: (1) Group 1 consisted of children who were still alive as of December 31, 1980 (live children). (2) Group 2 consisted of children who died during childhood (deaths between age 1 and 20 years, child deaths). As shown previously,[10] more than two thirds of these deaths were due to accidents. (3) Group 3 consisted of offspring who died before reaching age 1 year (infant deaths). As shown previously, the most common cause of death in this group was prematurity followed by congenital malformations.[10] For all children, information was abstracted from birth certificates obtained from the Pennsylvania State Health Department on birth variables including last menstrual period, parental ages, maternal parity, birth weight, gestational age, prenatal or delivery complications, and recorded congenital malformations. Information was abstracted from death records on causes of death.

Methods of analysis

Outcome variables. The length of gestation was computed by use of information on last menstrual period and birth date (number of completed weeks past last menstrual period). For about 13% of birth certificates, gestational age information could not be derived, and these children had to be excluded from the analysis. The length of gestation was analyzed as a continuous variable (mean gestational age in weeks) and also categorized into (1) preterm delivery ($\leq$36 weeks), (2) borderline maturity (37 to 38 weeks), and (3) term ($\geq$38 weeks). No instances of postterm delivery (>42 weeks) were recorded. Intrauterine growth was analyzed with birth weight used as a continuous variable as obtained on birth certificates (<2% of certificates had a missing birth weight). However, as birth weight is highly associated with gestational age, the distribution of birth weight by gestational age was used. Intrauterine growth was categorized into (1) IUGR, defined as a birth weight less than the tenth percentile for gestational age on the basis of sex- and gestational age–specific birth weight distribution of the total singleton births in the U.S. white population[13]; (2) mild growth delay (if birth weight for gestational age was between the tenth and twenty-fifth percentiles); (3) no growth delay (greater than the twenty-fifth percentile). In order to compute the intrauterine growth variable, information on gestational age had to be known. In the final analysis, only certificates with reported birth weight and gestational age were included (group 1, 183/208; group 2, 69/93; and group 3, 60/65).

Computation of inbreeding coefficients. The inbreeding coefficient of an individual represents the probability that the individual carries at an autosomal genetic locus two alleles that are identical by descent.[11] Inbreeding increases the frequency of recessive genotypes in the individual, which may be unrecognized in the heterozygous state but become deleterious in the homozygous state. Significant inbreeding effects on the incidence of a trait or disease indicate the presence of one or more autosomal recessive genes affecting the condition.[11] In this study, the inbreeding coefficients (F) of child, mother, and father were calculated by linking individuals with the computerized Amish genealogic registry[10] by means of unique family genealogic numbers. The registry is a complete ancestral history of present-day Amish of Lancaster County. It contains information on 8163 family entries with 42,465 offspring spanning more than 200 years and dating back to the pioneer migrants in the 1700s.[11] Inbreeding coefficients were calculated by use of the path method of identifying common ancestors as described elsewhere.[10]

Analysis of inbreeding effects. The effects of the inbreeding coefficients F of child, mother, and father on the length of gestation and the rate of intrauterine growth were studied. Analyses were conducted within each of the three groups. Inbreeding coefficients were used as continuous variables as well as categorized into four levels of increasing inbreeding: (1) $F < 1/256$ (farther than level of third cousins), (2) $1/128 > F \geq 1/256$ (corresponding to inbreeding between third cousin and second cousin once removed), (3) $1/64 > F \geq 1/128$ (corresponding to inbreeding between second cousin once removed and second cousin), and (4) $F \geq 1/64$ (corresponding to inbreeding closer than second cousin). Because first-cousin marriages are prohibited by religion, no direct first-cousin marriages are seen in this study population. It is to be noted that no child had an inbreeding coefficient equal to 0, which indicates the extent of close inbreeding in the Amish. Also only one mother's and three fathers' coefficients were equal to 0.

Univariate analyses included evaluation of the separate effects of child's, mother's, and father's F on mean gestational age and birth weight with the use of analysis

Table I. Birth weight/gestational age and inbreeding characteristics of the three groups in the Amish study

	Group 1: Live children (N = 183)	*Group 2: Child deaths (N = 60)*	*Group 3: Infant deaths (N = 69)*
Gestational age (wk) (mean ± SE)	39.6 ± 0.09	39.5 ± 0.19	36.1 ± 0.61*
<37 wk			
%	1.6	6.7	40.6†
No.	3	4	28
37-38 wk			
%	10.4	10.0	11.6
No.	19	6	8
Birth weight (gm) (mean ± SE)	3515 ± 32	3500 ± 68	2554 ± 120*
<10th percentile			
%	0.55	3.3	11.6†
No.	1	1.	8.
<25th percentile			
%	3.8	8.3	15.9†
No.	7	5	11
Child inbreeding‡ (mean ± SE)	114.2 ± 6.2	132.0 ± 13.0	151.1 ± 13.4*
Mother inbreeding‡ (mean ± SE)	126.1 ± 7.3	123.7 ± 13.2	132.9 ± 10.0
Father inbreeding‡ (mean ± SE)	122.8 ± 6.5	118.0 ± 13.7	114.6 ± 9.6

*$p < 0.05$ when compared with group 1 (t test).
†$p < 0.05$ when compared with group 1 (χ^2 test).
‡Inbreeding coefficients are multiplied by 10,000.

Table II. Mean gestational age and percent of children <37 weeks' and 37 to 38 weeks' gestation by child inbreeding level and group

	Child inbreeding level			
	$F \geq 1/64$	*$1/64 > F \geq 1/128$*	*$1/128 > F \geq 1/256$*	*$F < 1/256$*
Group 1: Live children (N = 183)				
N	42	67	47	27
Gestational age* (mean ± SE)	39.10 ± 0.25	39.70 ± 0.11	39.79 ± 0.15	39.93 ± 0.18
<37 wk†				
%	7.1	0	0	0
No.	3	0	0	0
37-38 wk†				
%	11.9	11.9	8.5	7.4
No.	5	8	4	2
Group 2: Child deaths (>1 yr) (N = 60)				
N	18	18	16	8
Gestational age (wk) (mean ± SE)	39.06 ± 0.40	39.56 ± 0.25	39.62 ± 0.44	40.25 ± 0.16
<37 wk				
%	11.1	0	12.5	0
No.	2	0	2	0
37-38 wk				
%	11.1	16.7	6.2	0
No.	2	3	1	0
Group 3: Infant deaths (<1 yr) (N = 69)				
N	27	18	13	11
Gestational age (wk) (mean ± SE)	37.81 ± 0.57	35.00 ± 1.4	35.54 ± 1.8	34.18 ± 1.6
<37 wk†				
%	29.6	50.0	38.5	54.6
No.	8	9	5	6
37-38 wk†				
%	14.8	0	7.7	27.3
No.	4	0	1	3

*$p < 0.05$ (simple linear regression of gestational age on inbreeding).
†$0.05 < p < 0.10$, χ^2 for trend (both groups combined).

Table III. Mean gestational age and percent of children <37 weeks' and 37 to 38 weeks' gestation by mother inbreeding level and group

	Mother inbreeding level			
	F ≥ 1/64	*1/64 > F ≥ 1/128*	*1/128 > F ≥ 1/256*	*F < 1/256*
Group 1: Live children (N = 183)				
N	53	60	48	22
Gestational age* (wk) (mean ± SE)	39.32 ± 0.21	39.65 ± 0.12	39.67 ± 0.14	40.14 ± 0.19
<37 wk†				
%	5.7	0	0	0
No.	3	0	0	0
37-38 wk†				
%	15.1	8.3	10.4	4.6
No.	8	5	5	1
Group 2: Child deaths (>1 yr) (N = 60)				
N	13	27	12	8
Gestational age* (wk) (mean ± SE)	38.92 ± 0.42	39.59 ± 0.22	39.50 ± 0.58	40.25 ± 0.41
<37 wk				
%	15.4	3.7	8.3	0
No.	2	1	1	0
37 to 38 wk				
%	15.4	7.4	8.3	12.5
No.	2	2	1	1
Group 3: Infant deaths (<1 yr) (N = 69)				
N	24	22	20	3
Gestational age (wk) (mean ± SE)	36.67 ± 1.07	36.77 ± 0.80	34.80 ± 1.35	34.67 ± 3.53
<37 wk				
%	29.2	40.9	50.0	66.4
No.	7	9	10	2
37-38 wk				
%	12.5	13.6	10.0	0
No.	3	3	2	0

*$p < 0.05$ (simple linear regression of gestational age on mother F).
†$p < 0.05$, χ^2 for trend (both groups combined).

of variance (to compare the four inbreeding levels) and simple linear regression (when F is used as a continuous measure). The incidences of prematurity and IUGR were compared between the four levels of inbreeding and χ^2 for trend was used to test for statistical significance of trends. However, because the levels of inbreeding of child, mother, and father may be correlated and other prenatal and birth-related factors may act as confounders, we used multivariate analysis to study the independent effects of child's, mother's, and father's F on mean gestational age (multiple linear regression) and the incidences of IUGR and prematurity (multiple logistic regression). Other factors considered in the multivariate analyses included sex, maternal and paternal ages, year of birth, birth order, sibship size, history of previous siblings who died in infancy, and father's occupation (farmer versus nonfarmer).

Finally, because prematurity and IUGR tend to be correlated, we used the log linear model,[15] which can incorporate both outcomes in the analysis and can examine the relationship of inbreeding among child, mother, and father with each of these outcomes after they are adjusted for one another and the relationship between the two outcomes. The log linear model is a useful technique to analyze the relationship among cross-classified data and is described in detail by Fienberg.[15] Essentially, the model fits a linear combination of parameters to derive an expected number of individuals in each cell. Parameters are estimated by use of the maximum likelihood procedure and can be interpreted in terms of odds ratios (similar to logistic regression). However, one main advantage over logistic regression is the ability to derive independent relationships among all variables in the model, including one or more dependent variables. Its disadvantage over the usual logistic model is that all variables have to be categorical.

Results

Characteristics of the three groups in the study. Table I shows birth weight and gestational age characteristics and inbreeding patterns of children in the three study groups. Generally, infant deaths (group 3) had a lower mean birth weight and gestational age and an increased frequency of IUGR when compared with children in the other two groups. However, few

Table IV. Mean gestational age and percent of children <37 weeks' and 37 to 38 weeks' gestation by father inbreeding level and group

	Father inbreeding level			
	$F \geq 1/64$	*$1/64 > F \geq 1/128$*	*$1/128 > F \geq 1/256$*	*$F < 1/256$*
Group 1: Live children (N = 183)				
N	53	56	48	26
Gestational age (wk) (mean ± SE)	39.72 ± 0.15	39.61 ± 0.15	39.38 ± 0.21	39.88 ± 0.16
<37 wk				
%	1.9	1.8	2.1	0
No.	1	1	1	0
37-38 wk				
%	3.8	14.3	16.7	3.9
No.	2	8	8	1
Group 2: Children deaths (>1 yr) (N = 60)				
N	15	17	18	10
Gestational age (wk) (mean ± SE)	39.73 ± 0.40	39.29 ± 0.44	39.44 ± 0.29	39.70 ± 0.33
<37 wk				
%	6.7	11.8	5.6	0
No.	1	2	1	0
37-38 wk				
%	6.7	5.9	11.1	20.0
No.	1	1	2	2
Group 3: Infant deaths (<1 yr) (N = 69)				
N	18	22	21	8
Gestational age (wk) (mean ± SE)	36.78 ± 1.12	35.41 ± 0.93	35.90 ± 1.30	36.75 ± 2.03
<37 wk				
%	33.3	50.0	38.1	37.5
No.	6	1	8	3
37-38 wk				
%	16.7	13.6	9.5	0
No.	3	3	2	0

children were born before 37 weeks (only three in group 1 and four in group 2). Likewise, very few children had IUGR (<tenth percentile) (only one in group 1 and one in group 2). Therefore our ability to detect statistical significance in these outcomes is limited by the small number of affected children. With respect to inbreeding characteristics, group 3 has a higher mean child inbreeding coefficient than group 1, which is consistent with earlier findings that inbreeding is a risk factor for infant mortality. The higher child F in group 2 than in group 1 is not statistically significant. Also both mean mother F and mean father F do not show significant differences among the three groups.

Unadjusted inbreeding effects on the length of gestation

Child inbreeding. Table II shows the unadjusted effects of child inbreeding levels on mean gestational age and the incidence of premature birth. In both groups 1 and 2, it can be seen that with increasing child inbreeding levels, there is a progressive decline in mean gestational age (a difference of almost 1 week between the two extremes) and an increase in the frequency of children born at <37 weeks' gestation and at 37 to 38 weeks' gestation (combined). However, trends in both groups were of borderline statistical significance ($0.05 < p < 0.10$). However, in group 3 (infant deaths), inbreeding is not associated with a reduction of mean gestational age. On the contrary, there is a suggestion of an opposite trend.

Maternal inbreeding. Table III shows the unadjusted effects of mother inbreeding levels on mean gestational age and the incidence of premature birth. Again, one notes in group 1 that with increasing maternal inbreeding levels, there is a progressive decline in mean gestational age and an increase in the frequency of children born at <37 weeks' gestation and at 37 to 38 weeks' gestation (combined). Findings in group 2 are generally consistent with those from group 1 and are statistically significant in the analysis of mean gestational age. However, in group 3 (infant deaths), inbreeding is not associated with a reduction of mean gestational age, again with a suggestion of an opposite trend (not significant).

Paternal inbreeding. Table IV shows the unadjusted effects of paternal inbreeding levels on mean gestational age and the incidence of premature birth. In all three groups, no significant effects of father's inbreeding coefficients can be seen.

Unadjusted inbreeding effects on intrauterine growth

Child inbreeding. Table V shows the unadjusted effects of child inbreeding levels on mean birth weight and the incidence of IUGR (less than tenth percentile) and

Table V. Mean birth weight and percent of children less than or equal to the tenth and twenty-fifth percentiles in weight for gestational age by child inbreeding level and group

	Child inbreeding level			
	F ≥ 1/64	*1/64 > F ≥ 1/128*	*1/128 > F ≥ 1/256*	*F < 1/256*
Group 1: Live children (N = 183)				
N	42	67	47	27
Birth weight (gm) (mean ± SE)	3410 ± 85	3516 ± 53	3561 ± 47	3595 ± 78
<10th percentile				
%	2.4	0	0	0
No.	1	0	0	0
<25th percentile				
%	7.1	3.0	2.1	3.7
No.	3	2	1	1
Group 2: Child deaths (>1 yr) (N = 60)				
N	18	18	16	8
Birth weight (gm) (mean ± SE)	3431 ± 138	3573 ± 116	3423 ± 152	3643 ± 100
<10th percentile				
%	11.1	0	0	0
No.	2	0	1	0
<25th percentile				
%	16.7	5.6	6.2	0
No.	3	1	1	0
Group 3: Infant deaths (<1 yr) (N = 69)				
N	27	18	13	11
Birth weight (gm) (mean ± SE)	2738 ± 133	2494 ± 276	2428 ± 319	2353 ± 362
<10th percentile				
%	11.1	0	23.1	18.2
No.	8	0	3	2
<25th percentile				
%	11.1	11.1	23.1	27.3
No.	3	2	3	3

mild growth delay (tenth to twenty-fifth percentile). It can be noted that in both groups 1 and 2 there is a lower mean birth weight and higher frequency of children born with IUGR (less than tenth percentile) and mild growth delay (tenth to twenty-fifth percentile) among those with a high level of inbreeding ($F \geq 1/64$) ($0.05 < p < 0.10$). Most of the difference, however, can be accounted by the mild growth delay group as there were very few children who were below the tenth percentile. In group 3, no child inbreeding effects can be seen.

Maternal inbreeding. Table VI shows the unadjusted effects of maternal inbreeding levels on mean birth weight and the incidence of IUGR (less than tenth percentile) and mild growth delay (tenth to twenty-fifth percentile). It can be noted that in both groups 1 and 2 there is a tendency for lower mean birth weight at high inbreeding levels ($F \geq 1/64$) but no difference in the frequency of children born at less than the twenty-fifth percentile at different inbreeding levels.

Paternal inbreeding. Table VII shows the unadjusted effects of paternal inbreeding levels on mean birth weight and the incidence of IUGR (less than tenth percentile) and mild growth delay (tenth to twenty-fifth percentile). No significant paternal inbreeding effects can be seen.

Multivariate analysis of inbreeding effects

Length of gestation. Table VIII shows the results of multiple linear regression analysis on mean gestational age and multiple logistic regression on the frequency of deliveries at ≤38 weeks. In this table, groups 1 and 2 are combined. However, similar results are obtained when they are analyzed separately. After simultaneous adjustment of child, mother, and father inbreeding coefficients as well as potential confounding factors (Table VIII), mother's F was found to be a strong predictor of both mean gestational age and the frequency of delivery at ≤38 weeks ($p < 0.001$). Predicted mean gestational ages vary from 39.9 weeks for $F = 0$ (no inbreeding) to 38.3 weeks for $F = 1/16$ (first cousins). Likewise, the predicted proportion of children born at ≤38 weeks varies from 6% for $F = 0$ to 70.1% for $F = 1/16$. However, despite these strong maternal inbreeding effects, it has to be noted the mother's F accounts for about 10% of the variance in gestational age, which suggests that other unknown factors are operating in predicting the length of gestation. On the other hand, child's F was found to be an independent predictor of mean gestational age ($p = 0.01$) but not the proportion of infants born at ≤38 weeks ($p = 0.311$) (Table VIII). The proportion of the variance in gestational age accounted for by the child's F was <5%.

Table VI. Mean birth weight and percent of children less than or equal to the tenth and twenty-fifth percentiles in weight for gestational age by mother inbreeding level and group

	Mother inbreeding level			
	$F \geq 1/64$	*$1/64 > F \geq 1/128$*	*$1/128 > F \geq 1/256$*	*$F < 1/256$*
Group 1: Live children (N = 183)				
N	53	60	48	22
Birth weight (gm) (mean ± SE)	3474 ± 68	3517 ± 50	3532 ± 59	3570 ± 105
<10th percentile				
%	1.9	0	0	0
No.	1	0	0	0
<25th percentile				
%	3.8	3.3	6.3	0
No.	2	2	3	0
Group 2: Child deaths (>1 yr) (N = 60)				
N	13	27	12	8
Birth weight* (gm) (mean ± SE)	3234 ± 147	3601 ± 80	3426 ± 185	3700 ± 215
<10th percentile				
%	7.7	0	8.3	0
No.	1	1	1	0
<25th percentile				
%	7.7	3.7	8.3	25.0
No.	1	1	1	2
Group 3: Infant deaths (<1 yr) (N = 69)				
N	24	22	20	3
Birth weight (gm) (mean ± SE)	2691 ± 213	2678 ± 180	2247 ± 239	2604 ± 735
<10th percentile				
%	12.5	9.1	15.0	0
No.	3	2	3	0
<25th percentile				
%	12.5	9.1	30.0	0
No.	3	2	6	0

*p <0.05 (simple linear regression of birth weight on mother F).

Therefore, although child inbreeding could be an independent predictor of the length of gestation, our results are equivocal and cannot be confirmed in both types of analyses. Last, not shown in Table VIII is that paternal inbreeding exerted no independent effects on mean gestational age and the risk of early delivery (≤38 weeks).

Intrauterine growth. Table IX shows results of multiple logistic regression analysis on the incidence of intrauterine growth delay (less than twenty-fifth percentile) in groups 1 and 2 combined. However, similar results are obtained when they are analyzed separately. After simultaneous adjustment of child, mother, and father inbreeding coefficients as well as potential confounding factors (Table IX), only child's F was found to be an independent predictor for the risk of being below the twenty-fifth percentile.

Simultaneous analysis of IUGR and prematurity. Fig. 1 shows the results of the log linear analysis of length of gestation (≤38 weeks as outcome) and intrauterine growth (less than twenty-fifth percentile as outcome) in relation to child, maternal, and paternal inbreeding (≥1/64) in groups 1 and 2 combined. Values in Fig. 1 correspond to adjusted odds ratios relating different variables. *Solid lines* correspond to relationships that are statistically significant at the 0.05 level. This analysis indicates that: (1) child inbreeding is associated with a 3.02-fold increase in the risk of being born below the twenty-fifth percentile in weight for gestational age, (2) maternal inbreeding is associated with a 2.77-fold increase in the risk of being born at ≤38 weeks, (3) paternal inbreeding is not a risk factor for either outcomes, (4) there is an association between short gestation and intrauterine growth delay (odds ratio = 3.48), and (5) maternal inbreeding is associated with child inbreeding (odds ratio = 1.90). It is to be noted that the above relationships were not changed by introducing other variables in the model (considered in Tables VIII and IX). Also, although child inbreeding was associated with a 1.65-fold increase in the risk of short gestation, this increase was not statistically significant.

Comment

This study suggests the presence of autosomal recessive genes affecting the length of gestation and intrauterine growth in the Old Order Amish. The main finding of the study, however, is the differential impact of the genetic component on prematurity and IUGR.

Table VII. Mean birth weight and percent of children less than or equal to the tenth and twenty-fifth percentiles in weight for gestational age by father inbreeding level and group

	Father inbreeding level			
	F ≥ 1/64	*1/64 > F ≥ 1/128*	*1/128 > F ≥ 1/256*	*F < 1/256*
Group 1: Live children (N = 182)				
N	53	56	48	26
Birth weight (gm) (mean ± SE)	3623 ± 55	3444 ± 52	3381 ± 65	3695 ± 95
<10th percentile				
%	0	1.8	0	0
No.	0	1	0	0
<25th percentile				
%	1.9	7.1	2.1	3.8
No.	1	4	1	1
Group 2: Child deaths (>1 yr) (N = 60)				
N	15	17	18	10
Birth weight (gm) (mean ± SE)	3428 ± 128	3452 ± 160	3630 ± 91	3455 ± 184
<10th percentile				
%	0	11.8	0	0
No.	1	2	0	0
<25th percentile				
%	7.7	11.8	5.6	10.0
No.	1	2	1	1
Group 3: Infant deaths (<1 yr) (N = 69)				
N	18	22	21	8
Birth weight (gm) (mean ± SE)	2678 ± 206	2496 ± 198	2519 ± 247	2518 ± 436
<10th percentile				
%	5.6	4.6	14.3	37.5
No.	1	1	3	3
<25th percentile				
%	11.1	4.6	23.8	37.5
No.	2	1	5	3

Table VIII. Multivariate analysis of the effects of child and mother inbreeding on mean gestational age and the proportion of infants ≤38 weeks' gestation (groups 1 and 2 combined)

	Mean gestational age (wk)		*≤38 wk (%)*	
Level of inbreeding	*Predicted**	*95% Confidence limits*	*Predicted†*	*95% Confidence limits*
Child inbreeding‡				
F = 0	39.86	Reference	9.3	Reference
F = 1/256	39.77	30.68-39.85	10.0	8.7-11.5
F = 1/128	39.68	39.54-39.82	10.8	8.1-14.2
F = 1/64	39.51	39.24-39.78	12.4	7.1-20.9
F = 1/32	39.16	38.62-39.70	16.4	5.3-40.5
F = 1/16	38.46	37.37-39.54	27.1	3.0-81.8
Mother inbreeding‡				
F = 0	39.93	Reference	6.0	Reference
F = 1/256	39.82	39.76-39.88	7.4	6.5-8.3
F = 1/128	39.72	39.60-39.84	9.1	6.1-11.5
F = 1/64	39.51	39.27-39.75	13.5	8.5-20.8
F = 1/32	39.09	38.61-39.58	27.9	12.1-52.1
F = 1/16	38.26	37.30-39.22	70.1	22.8-94.9

*Predicted means were obtained by use of multiple linear regression analysis with gestational age as dependent variable. Independent variables included child, mother, and father inbreeding coefficients (continuous), maternal and paternal ages, birth order, year of birth, father's occupation, sibship history of death, total number of siblings, sex, and growth retardation (less than or equal to the twenty-fifth percentile).

†Predicted percents were obtained by use of multiple logistic regression analysis with binary outcomes ≤38 weeks' versus >38 weeks' gestation. The independent variables are the same ones used in multiple lienar regressions.

‡Child inbreeding effect: $p = 0.01$ on mean gestational age and $p = 0.311$ (NS) on percent ≤38 weeks. Mother inbreeding effect: $p < 0.001$ on mean gestational age and $p < 0.001$ on percent ≤38 weeks.

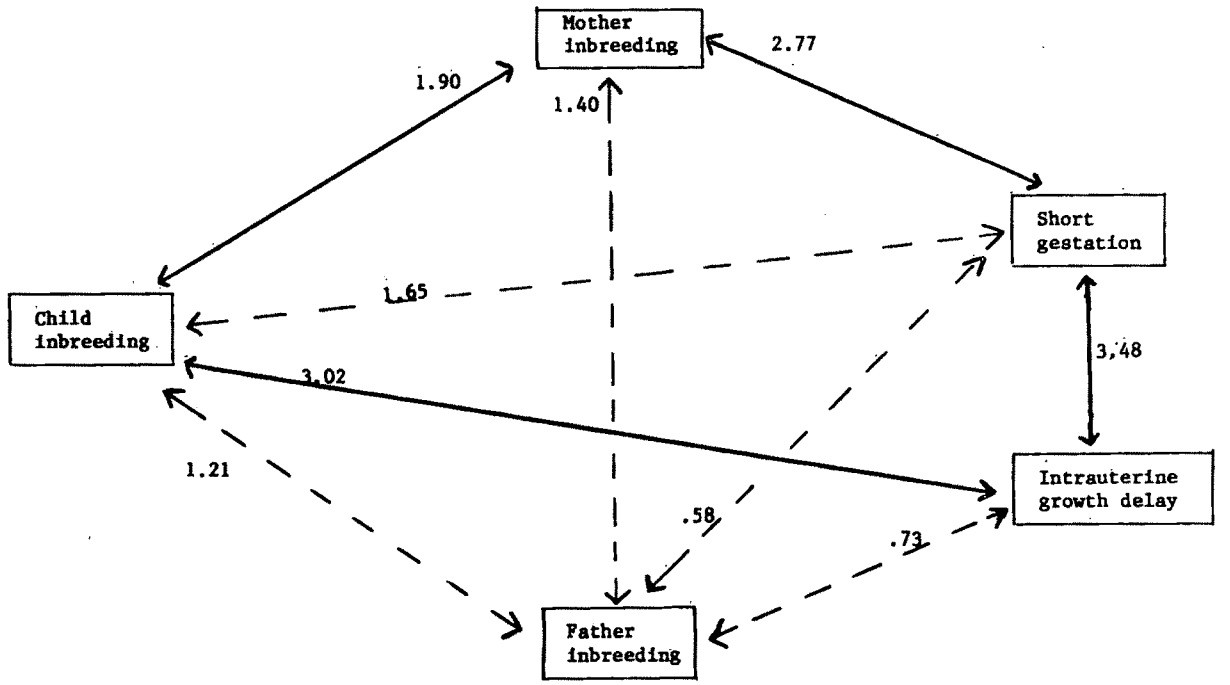


Fig. 1. Log linear analysis of the effects of child, mother, and father inbreeding on gestational age and intrauterine growth. Inbreeding variables were categorized into close inbreeding (≥1/64) versus distant inbreeding (<1/64). Short gestation defined as ≤38 weeks. Intrauterine growth delay defined as less than the twenty-fifth percentile in birth weight for gestational age. Numbers represent adjusted odds ratios relating each two variables. *Solid lines* represent relationships that are significant at the 0.05 level, whereas *dotted lines* represent nonsignificant relationships.

Table IX. Multivariate analysis of the effects of child and mother inbreeding on percent of children less than the twenty-fifth percentile for gestational age (groups 1 and 2 combined)

Level of inbreeding	*<25th percentile (%)*	
	*Predicted**	*95% Confidence interval*
Child inbreeding†		
F = 0	2.1	Reference
F = 1/256	2.7	2.1-3.2
F = 1/128	3.3	2.2-4.9
F = 1/64	5.0	2.2-11.0
F = 1/32	11.4	2.3-41.6
F = 1/16	43.5	2.5-95.9
Mother inbreeding†		
F = 0	3.6	Reference
F = 1/256	3.8	3.0-4.7
F = 1/128	3.9	2.5-6.0
F = 1/64	4.2	1.7-9.9
F = 1/32	4.9	1.8-24.1
F = 1/16	6.5	1.8-72.8

*Predicted percents less than the twenty-fifth percentile were obtained by use of multiple logistic regression analysis with a binary outcome (less than twenty-fifth percentile). Independent variables include child, mother, and father inbreeding coefficients (continuous) and other factors considered in Table VIII.

†Child inbreeding effect: p = 0.05. Mother inbreeding effect: p = 0.745 (NS).

The data suggest that while the maternal genotype is associated with a shorter gestation time (as reflected by the maternal inbreeding effect), the fetal genotype is associated with retardation in intrauterine growth (as reflected in the child inbreeding effect). There is a suggestion that the fetal genotype may also be associated with premature birth. However, this effect is not consistent in different analyses and should be confirmed in other studies. Also, the lack of any child or maternal inbreeding effects in group 3 is not entirely surprising since infant deaths constitute a highly selected sample representing less than 2% of all Amish births.[10] In such infants, other factors such as congenital malformations and pregnancy and labor complications are causally associated with both prematurity and IUGR and can mask any inbreeding effects.

The study has several potential limitations. First, birth certificates are known to be incomplete in ascertainment of gestational age. Also, the reliance on last menstrual period is subject to maternal recall and may introduce errors in computation of gestational age. In this study, about 13% of certificates had to be excluded because of unknown gestational age information. However, this can affect our findings only if there is a bias in collecting gestational age data depending on inbreeding levels of mother and child. The existence of such a bias is highly unlikely given the relative cultural

and health care homogeneity of the Old Order Amish. Second, because very few infants met the usual criteria for prematurity (<37 weeks) and IUGR (less than tenth percentile), we had to extend the analysis to include outcomes such as borderline maturity (37 to 38 weeks) and mild growth delay (tenth to twenty-fifth percentile). We view this extension as reasonable given the consistency of findings between the analysis of mean gestational age and the binary variable and also because intrauterine growth can be considered as a continuum rather than a totally discrete entity. Moreover, Johnstone and Inglis[7] found that familial trends in IUGR apply for both the tenth and twenty-fifth percentile cutoffs. Third, because the Amish are highly inbred, the variation in inbreeding coefficient values is limited. For example, no child had an F = 0, and no child was an offspring of first-cousin marriage (F = 1/16). However, this lack of variation of inbreeding values, if anything, tends to reduce the statistical power needed to detect true differences between groups and lead to more conservative findings. Also, a reduction in power will be noted when inbreeding coefficients are used as categorical variables (F > 1.64 versus <1/64) as required by the log linear model. This is not a problem in this study since similar results were obtained in linear and logistic analyses with F values used as continuous or categorical variables.

The significance of this analysis can be viewed along several lines. First, because the Amish have homogeneous characteristics, inbreeding effects are unlikely to be confounded by other factors that may be associated with both inbreeding and low birth weight and, we believe, reflect a true genetic etiology. The lack of adjustment for confounding factors has been a longstanding problem in the interpretation of inbreeding effects in many studies.[10] Since low socioeconomic status tends to be associated with inbreeding in various populations, it needs to be adjusted for before effects of inbreeding on child morbidity and mortality are interpreted. Such an issue has often been overlooked, especially in the early literature on inbreeding.[10] Second, this study is one of few that have analyzed the effects of parental inbreeding in addition to the child inbreeding on disease outcomes. Parental inbreeding information is usually not available in most populations because it requires knowledge of pedigree structure beyond the parents. However, such data are readily available in the Amish multigenerational pedigrees. Third, most studies dealing with genetic analysis of birth weight and with inbreeding effects on birth weight have not examined prematurity and IUGR simultaneously or have relied exclusively on separate analyses of birth weight and gestational age.

How does this analysis compare with other studies of inbreeding effects? Generally, no effect of child inbreeding on gestational age has been found in several studies (see reference 16). However, analysis of inbreeding effects on birth weight has given equivocal results. In Japanese live births, Morton[16] found that child inbreeding had a significant depressing effect on birth weight, which was considered a reflection of intrauterine growth. Other studies found no significant effect of child inbreeding on birth weight. These equivocal findings can be partly attributed to the failure of examining the gestational age component of birth weight. If inbreeding affects intrauterine growth but not the length of gestation, its effect on birth weight may be masked by the proportion of premature infants among those with low birth weight. Another reason for the discrepancies in findings may be related to the extent and duration of inbreeding in the population. With prolonged and intense inbreeding (for example, in India), there may be lowering of equilibrium allele frequencies for lethal genes,[11] thus leading to a smaller observed effect of inbreeding on IUGR (which is associated with a higher risk of death). On the other hand, several analyses of parental inbreeding effects in Japan found no significant maternal or paternal inbreeding effect on prereproductive mortality or growth and development throughout childhood.[10] However, we are not aware of any published parental inbreeding analysis of gestational age and intrauterine growth.

Differences in the role of genetic factors in the etiology of prematurity and IUGR as suggested in this article are also consistent with several analyses that showed a specificity in familial aggregation in prematurity and IUGR. Women who had one infant with IUGR seem to be at higher risk of having another with IUGR but not with prematurity, while women with a premature infant seem to have a higher risk for prematurity in a subsequent pregnancy but not IUGR (see reference 7). However, although these analyses point to the persistence of etiologic factors operating in successive pregnancies, they cannot infer whether such factors are genetic or environmental in nature. Our study shows that the basis for the specificity in familial recurrence is due, at least in part, to a differential genetic component acting on prematurity and IUGR, respectively. Finally, this study reemphasizes the need to consider both prematurity and IUGR in genetic and epidemiologic studies of low birth weight. This will lead to specific characterization of risk profiles associated with one or both entities and ultimately to improved prevention strategies.

REFERENCES

1. McCormick MC. The contribution of low birth weight to infant mortality and childhood morbidity. N Engl J Med 1985;312:82-90.
2. Alberman E. Low birthweight. In Bracken MB, ed. Perinatal epidemiology. New York: Oxford University Press, 1984:86-98.

3. Hemminki E, Starfield B. Prevention of low birth weight and preterm birth. Literature review and suggestions for research policy. Milbank Mem Fund Q 1978;56:339-61.
4. Starfield B, Shapiro S, McCormick M, Bross D. Mortality and morbidity in infants with intrauterine growth retardation. J Pediatr 1982;101:978-83.
5. Villar J, Khoury MJ, Finucane FF, Delgado HL. Differences in the epidemiology of prematurity and intrauterine growth retardation. Early Hum Dev (in press).
6. Morton NE. The inheritance of human birth weight. Ann Hum Genet 1955;20:125-34.
7. Johnstone F, Inglis L. Familial trends in low birth weight. Br Med J 1974;3:659-61.
8. Seeds JW. Impaired fetal growth: definition and clinical diagnosis. Obstet Gynecol 1984;64:303-10.
9. Goldenberg RL, Nelson KG, Koski JF, Cutter GR. Low birth weight, intrauterine growth retardation, and preterm delivery. Am J Obstet Gynecol 1985;152:980-4.
10. Khoury MJ. A genealogic study of inbreeding and prereproductive mortality in the Old Order Amish [Dissertation]. Baltimore: The Johns Hopkins University, 1985.
11. Egeland JA. Descendants of Christian Fisher and other Mennonite pioneer families. Baltimore: Moore Clinic, The Johns Hopkins Hospital, 1972.
12. Khoury MJ, Cohen BH, Diamond EL, Chase GA, McKusick VA. Inbreeding and prereproductive mortality in the Old Order Amish. III. Direct and indirect effects of inbreeding. Am J Epidemiol 1987;125:473-83.
13. Hoffman HJ, Stark CR, Lundin FE, Ashbrook JD. Analysis of birth weight, gestational age and fetal viability, US births 1968. Obstet Gynecol Surv 1974;29:651-81.
14. Cavalli-Sforza LL, Bodmer WF. The genetics of human populations. San Francisco: WH Freeman, 1971:341-86.
15. Fienberg SE. The analysis of cross classified categorical data. Cambridge, Massachusetts: MIT Press, 1983.
16. Morton NE. Empirical risks in consanguineous marriages: birth weight, gestation time, and measurements of infants. Am J Hum Genet 1959;10:344-9.

A more comprehensive list of references is available from the authors on request.

Fetal weight estimation formulas with head, abdominal, femur, and thigh circumference measurements

Anthony M. Vintzileos, M.D., Winston A. Campbell, M.D., John F. Rodis, M.D., Roy Bors-Koefoed, M.D., and David J. Nochimson, M.D.

Farmington, Connecticut

Most current sonographic techniques for estimating fetal weight involve measurements of the head, abdomen, and femur length both alone and in combination. The value of the fetal thigh circumference measurement in addition to the head, abdominal, and femur length measurements has not been investigated previously. Eighty-nine patients were scanned within 72 hours of delivery, and the following ultrasonic fetal parameters were obtained in all fetuses: biparietal diameter, head circumference, abdominal circumference, femur length, and thigh circumference. With multiple stepwise regression analysis, the best-fit formulas were developed with one, two, three, four, and five parameters, respectively. The best results were obtained by combining measurements of all five parameters: biparietal diameter, head circumference, abdominal circumference, femur length, and thigh circumference. The mean error of this formula was 6%, the mean deviation 0.3%, and the SD 7.8%. These data suggest that the addition of thigh circumference to measurements of the head, abdomen, and femur length improves the accuracy of fetal weight estimates. (Am J Obstet Gynecol 1987;157:410-4.)

Key words: Estimated fetal weight, thigh circumference

Initial attempts to estimate fetal weight by ultrasound were made on the basis of measurements of individual fetal parameters such as the biparietal diameter (BPD)[1] or abdominal circumference.[2] Weight estimates obtained with abdominal circumference alone have been reported to have an SD between 11.1% and 11.9%.[3] Subsequent reports demonstrated that the accuracy of estimating fetal weight is improved when multiple fetal parameters are used.[3-6] The methods that give reasonably accurate results and are the simplest to apply are those based on the use of two parameters, namely abdominal circumference in combination with the BPD[4, 5] or abdominal circumference in combination with the femur length.[3, 6, 7] Weight estimates obtained with the use of two parameters have been reported to have an SD between 8.2% and 12.9%.[3] Hadlock et al.[3]

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Connecticut Health Center.
Received for publication August 29, 1986; revised March 9, 1987; accepted March 13, 1987.
Reprint requests: Anthony M. Vintzileos, M.D., Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Connecticut Health Center, 263 Farmington Ave., Farmington, CT 06032.

reported an improvement in the fetal weight estimates within 7.5% (1 SD) of the actual birth weight by combining measurements of the head (BPD and head circumference), abdomen (abdominal circumference), and femur (length). The better correlation to the actual birth weight with the use of the femur length in addition to head and abdominal measurements was attributed to the strong linear relationship between femur length and crown-heel length.

Because the fetal weight depends not only on head and body dimensions but also on extremity dimensions, it is reasonable to investigate the role of thigh circumference measurement in improving fetal weight estimates. The range of normal values for fetal thigh circumference measurements from 20 to 40 weeks of gestation has been previously reported by our group.[8] The present study was undertaken to determine whether the addition of the thigh circumference to the measurements of the head, abdomen, and femur length could improve the accuracy of in utero fetal weight estimates.

Material and methods

The study included 89 pregnant women. The majority of the patients were transferred to the University of Connecticut Health Center because of preterm labor or other medical complications of pregnancy (i.e., pregnancy-induced hypertension, vaginal bleeding, etc.). The included patients had singleton pregnancies, and in all cases clinical and ultrasound dating (BPD, femur length) were in agreement. The gestational ages were confirmed by Dubowitz scores[9] after birth. All patients delivered within 72 hours of the ultrasound evaluation. Infants with congenital anomalies were excluded.

The ultrasonic measurements were made with a linear array real-time ultrasound (SRT, General Electric Medical Systems Group, Milwaukee, Wisconsin) equipped with a 3.5 MHz transducer. Ultrasound velocity was 1540 m/sec. Freeze-frame capability was available, and on-screen calibers were used for the measurements. The included patients had all five parameters measured: BPD, head circumference, abdominal circumference, femur length, and thigh circumference. The fetal BPD was determined at the level of the thalami and was measured from leading edge to leading edge (outer to inner skull table). The head circumference was determined at the level of the BPD with measurements from outer to outer margins. The abdominal circumference was measured perpendicular to the long axis of the fetal body at the level of the umbilical vein–ductus venosus complex; measurements were obtained from outer to outer border. The femur length was measured according to the technique of O'Brien and Queenan.[10] The thigh circumference was measured according to the technique of Vintzileos et al.[8] The long axis of the femur was first imaged; the transducer was then rotated 90 degrees to obtain a cross-sectional profile of the middle of the thigh at a position that the bone profile was as round as possible and the boundary of the thigh profile was well defined. All circumferences (head, abdomen, thigh) were determined with the formula $D_1 + D_2 \times 1.57$ on the basis of two diameters at right angles to one another. This technique has been demonstrated to give results equivalent to those obtained with a map measurer.[11] In the present study we chose this method to calculate circumferences because the use of diameter measurements and the equation for the circumference of a circle has been shown to result in a more accurate estimated fetal weight than when a map measurer is used.[12] All ultrasound measurements were expressed in centimeters and the birth weights in grams.

As in previous studies,[3-7] fetal weights were best estimated as a logarithmic function of the ultrasonically measured fetal parameters. Polynomial expressions of the fetal parameters were considered up to the fourth degree. All possible interactions of the fetal parameters were considered up to the third degree. The regression analyses calculated were polynomial regressions with the use of one of the parameters at a time and stepwise regressions that considered all five parameters, the powers of each parameter, and all interaction terms of the five parameters.[13] Results included the best models to predict birth weights with one, two, three, four, and five fetal parameters. The accuracy of the models was examined by the mean residual (predicted birth weight minus actual birth weight), the SEM residual, mean absolute residual (absolute value of the residuals), the SEM absolute residual, and the mean percent error. The mean deviation and the SD for each model were also determined according to previously described techniques.[3, 14] In this context the mean deviation is a measure of systematic errors and the SD is a measure of random errors.

Results

The actual birth weights of the 89 newborn infants ranged from 570 to 4678 gm (mean, 2328 gm), and gestational ages ranged from 24 to 42 weeks (mean, 34.9 weeks). BPD measurements ranged from 5.8 to 10.2 cm (mean, 8.2 cm), head circumference measurements from 21.1 to 35 cm (mean, 30.1 cm), abdominal circumference from 18.8 to 38.8 cm (mean, 29.3 cm), femur length from 4.0 to 8.5 cm (mean, 6.5 cm), and thigh circumference from 7.7 to 18.5 cm (mean, 13.1 cm).

The best five models with one, two, three, four, and five parameters are shown in Table I. The best model with one ultrasonically measured parameter used the

Table I. Fetal weight estimation formulas

Model	*Fetal parameters*	*Regression equation**
1	AC	Log_{10} BW = 3.046 − 0.18(AC) + 0.015(AC)^2 − 0.0004(AC)^3 + 0.000004(AC)^4
2	AC, BPD	Log_{10} BW = 1.879 + 0.026(AC) + 0.084(BPD)
3	HC, FL, AC	Log_{10} BW = 2.082 + 0.004(HC × FL) + 0.0018(AC) − 0.00000001509(HC × FL)^3
4	AC, BPD, FL, TC	Log_{10} BW = 1.897 + 0.015(AC) − 0.057(BPD) + 0.054(FL) + 0.011(TC)
5	HC, FL, AC, BPD, TC	Log_{10} BW = 2.144 + 0.004(HC × FL) + 0.014(AC) − 0.00000001983(HC × FL)^3 + 0.000006(BPD × TC)^2

AC = Abdominal circumference; BPD = biparietal diameter; HC = head circumference; FL = femur length; TC = thigh circumference; BW = birth weight.

*Weight in grams and measurements in centimeters.

Table II. Comparison of fetal weight estimation formulas

Model	R^2	*Mean residual*	*SEM residual*	*Mean absolute residual*	*SEM absolute residual*	*Mean percent error*	*Mean deviation* ± SD (%)*
1	0.9404	−13.95	30.94	216.83	18.90	9.41	0.6 ± 11.4
2	0.9501	−2.40	29.31	191.59	19.23	8.20	0.5 ± 10.6
3	0.9633	−9.29	26.56	167.41	18.25	6.97	0.3 ± 8.8
4	0.9641	−0.15	23.72	163.26	15.21	7.24	0.3 ± 8.9
5	0.9716	−6.12	21.97	137.51	14.40	6.00	0.3 ± 7.8

*Deviation (%) = Mean of predicted weight minus actual weight per actual weight times 100.

Table III. Comparison of fetal weight estimation formulas on the basis of the percentage of cases that the prediction is within ±5% and ±10% of the actual weight

Model	*Percent within 5% of actual weight*	*Percent within 10% of actual weight*
1	26.6	60.7
2	39.5	69.7
3	42.1	75.0
4	32.9	76.3
5	54.0	80.3

abdominal circumference (model 1); the best model with two parameters used a combination of BPD and abdominal circumference (model 2); the best model with three parameters used a combination of head circumference, abdominal circumference, and femur length (model 3); the best model with four parameters used a combination of BPD, abdominal circumference, femur length, and thigh circumference (model 4); the best model with five parameters used a combination of BPD, head circumference, abdominal circumference, femur length, and thigh circumference (model 5).

The accuracy of these five models is illustrated in Table II. On the basis of the definition of residual, the positive residual is indicative of overestimation and a negative residual is indicative of underestimation. As can be seen in Table II, the best results were obtained with model 5 (R^2, 0.9716; mean residual, −6.12; SEM residual, 21.97; mean absolute residual, 137.51; SEM absolute residual, 14.40; mean error 6%; mean deviation, 0.3%; and SD, 7.8%).

The percentage of cases in which the estimated fetal weight was within ±5% and ±10% of the actual birth weight was calculated on the basis of these five models, and the results are shown in Table III. Model 5 gave the best results predicting the actual weight within ±5% in 54% of the cases and within ±10% in 80.3% of the cases.

The data were also analyzed according to actual birth weight groups. The accuracy of the models according to birth weight groups is demonstrated in Table IV. As can be seen in the birth weight group of <1500 gm, all five models gave a systematic overestimation. The mean deviations for models 1-5 were 7.4%, 8.7%, 5%, 7.2%, and 4.7%, respectively. In the birth weight group between 1500 to 2500 gm, all five models gave a systematic underestimation. The mean deviations for models 1-5 were −0.5%, −4.2%, −1.1%, −3.9%, and −1.5%, respectively. For the birth weight group of >2500 gm, models 2 and 4 gave a systematic overestimation (mean deviation, 1.4% for both models), and models 1, 3, and 5 gave a systematic underestimation (mean deviations, −1.6%, −0.3%, and −0.03%, respectively). The variability of the weight estimates (SD) obtained with model 5 was considerably less than that seen with the other models; the SD for weights <1500 gm was 8.7%, SD for weights between 1500 and 2500 gm was 6.4%, and SD for weights >2500 gm was 8.1%.

The analysis of the results of the present study was conducted in accordance with the format of previous investigations[1-7, 12, 14] to permit meaningful comparisons. As such, fetal gender was not considered as a variable in the development of the fetal weight estimation for-

Table IV. Comparison of fetal weight estimation formulas according to birth weight groups

	Model 1	*Model 2*	*Model 3*	*Model 4*	*Model 5*
Mean residual					
S	88.89	88.56	61.98	72.75	56.15
A	−3.38	−85.70	−15.97	−76.92	−24.75
L	−80.43	38.21	−38.03	42.73	−18.01
SEM residual					
S	36.03	35.86	26.98	26.27	26.49
A	36.89	27.84	26.80	24.53	23.31
L	66.48	63.58	59.68	50.12	48.26
Mean absolute residual					
S	126.45	115.95	91.45	93.18	84.08
A	170.03	129.78	109.84	127.01	100.44
L	316.51	293.26	264.88	235.75	202.53
SEM absolute residual					
S	27.55	29.76	20.13	21.17	20.54
A	21.39	21.42	18.01	16.16	15.08
L	34.48	33.57	34.53	25.67	30.43
Mean percent error					
S	11.53	11.07	8.21	9.28	7.65
A	8.33	6.18	5.41	6.19	4.98
L	9.46	8.86	7.95	7.31	6.22
Mean deviation ± SD (%)					
S	7.4 ± 13.0	8.7 ± 12.2	5.0 ± 8.8	7.2 ± 9.4	4.7 ± 8.7
A	−0.5 ± 10.1	−4.2 ± 7.0	−1.1 ± 7.3	−3.9 ± 6.4	−1.5 ± 6.4
L	−1.6 ± 10.9	1.4 ± 10.3	−0.3 ± 9.6	1.4 ± 8.6	−0.03 ± 8.1

S = <1500 gm (26 patients); A = 1500 to 2500 gm (33 patients); L = >2500 gm (30 patients).

mulas. In addition, the fetal gender may not be known before birth in many cases. Because the knowledge of fetal gender may improve the accuracy of weight estimates, it would be of interest to investigate its role in future studies.

Comment

The results of the present study indicate that the accuracy of estimating fetal weight by ultrasound is improved when multiple fetal parameters are used. The best model with one ultrasonically determined parameters was with the use of abdominal circumference (model 1). This observation is in agreement with the findings of others[2-4] who have also demonstrated that abdominal circumference is the single best index of fetal weight. According to Hadlock et al.,[3] different models that use abdominal circumference alone have an SD between 11.1% and 11.9%. Our model (model 1), which used abdominal circumference alone, was also found to have the same variability of the weight estimates (SD, 11.4%). According to our data, the best model with the use of two ultrasonically determined parameters was the one that used the BPD and abdominal circumference. Models that used BPD and abdominal circumference have been shown to be reasonably accurate and the simplest to apply, since the estimated fetal weight is read directly from tables that have been prepared with computer assistance.[4, 5] In the study by Hadlock et al.,[3] models that used head measurements (BPD or head circumference) in conjunction with abdominal circumference had an SD between 8.7% and 12.9%. Our results with the two best parameters (BPD and abdominal circumference, model 2) were similar in terms of variability of the weight estimates (SD, 10.6%). The model used by Hadlock et al.,[3] which used femur length and abdominal circumference, had a SD of 8.2%.

With three parameters (BPD or head circumference in conjunction with abdominal circumference and femur length), the variability of the weight estimates has been shown[3] to be improved (1 SD 7.6% to 7.7%); model 3 in our present study, which uses three parameters (head circumference, abdominal circumference, and femur length), was also found to improve accuracy as compared with our models 1 and 2. In addition, our results with three parameters were very similar with the data from Hadlock et al.[3]

The accuracy of the use of four parameters (BPD, head circumference, abdominal circumference, and femur length) in the study by Hadlock et al.[3] was not improved substantially as compared with their models that used three parameters; in that study the SD of models that used these four parameters was 7.5%. In our study in the model that used the best four parameters (model 4) the thigh circumference entered the equation; the SD of this model was 8.9%, very similar to that of model 3.

The best model was the one that used all five parameters (model 5). This model had an SD of 7.8%. The accuracy of this model is illustrated in Tables II-IV. In weights <1500 gm, this model gave a systematic overestimation (mean deviation, 4.7%); in gestations be-

tween 1500 and 2500 gm, the model gave a systematic underestimation (mean deviation, −1.5%); and in weights >2500 gm, the model gave a systematic underestimation (mean deviation, −0.03%).

One fetal weight estimation formula that used thigh circumference measurement has been previously presented by Hohler et al.[15] These investigators constructed a formula that used thigh circumference in conjunction with abdominal circumference and femur length to estimate fetal weight. They found that this formula was more accurate than the equation by Warsof et al.[4] and as accurate as the one by Shepard et al.[5]; the investigators recommended the use of this formula when the BPD is unobtainable or when the cephalic index is not within normal limits.

Formulas incorporating thigh circumference measurements may be proven most useful in predicting fetal weight when growth abnormalities are present. Fetal growth aberrations (intrauterine growth retardation or macrosomia) are associated with changes in soft tissue mass, which is decreased in intrauterine growth retardation and is excessive in macrosomia. Pediatric experience has shown that thigh circumference is one of the parameters that reflects soft tissue mass.[16] Our group[8] has also shown that the ultrasonically determined thigh circumference is decreased in fetuses with intrauterine growth retardation and increased in fetuses with macrosomia. It is our opinion that thigh circumference formulas may be helpful in improving the accuracy of fetal weight estimates in growth-retarded and macrosomic fetuses who have quantitative disturbances in the soft tissue mass. The accuracy of this formula in predicting weight in fetuses with normal and abnormal growth is being currently evaluated prospectively at the University of Connecticut Health Center.

REFERENCES

1. Ianniruberto A, Gibbons JM. Predicting fetal weight by ultrasound B scan cephalometry: an improved technique with disappointing results. Obstet Gynecol 1971;37:689.
2. Campbell S, Wilkin D. Ultrasonic measurement of the fetal abdominal circumference in estimation of fetal weight. Br J Obstet Gynaecol 1975;82:689.
3. Hadlock FP, Harrist RB, Carpenter RJ, et al. Sonographic estimation of fetal weight. The value of femur length in addition to head and abdomen measurements. Radiology 1984;150:535.
4. Warsof SL, Gohari P, Berkowitz RL, et al. The estimation of fetal weight by computer-assisted analysis. AM J OBSTET GYNECOL 1977;128:881.
5. Shepard MJ, Richards VA, Berkowitz RL, et al. An evaluation of two equations for predicting fetal weight by ultrasound. AM J OBSTET GYNECOL 1982;142:47.
6. Warsof SL, Wolf P, Coulehan J, et al. Comparison of fetal weight estimation formulas with and without head measurements. Obstet Gynecol 1986;67:569.
7. Campbell WA, Vintzileos AM, Neckles S, et al. Use of the femur length to estimate fetal weight in premature infants: preliminary results. J Ultrasound Med 1985;4:583.
8. Vintzileos AM, Neckles S, Campbell WA, et al. Ultrasound fetal thigh-calf circumferences and gestational age-independent fetal ratios in normal pregnancy. J Ultrasound Med 1985;4:287.
9. Dubowitz LMS, Dubowitz O, Goldberg C. Clinical assessment of gestational age in the newborn infant. J Pediatr 1970;77:1.
10. O'Brien GD, Queenan JT. Growth of the ultrasound fetal femur length during normal pregnancy. Part I. AM J OBSTET GYNECOL 1981;141:833.
11. Hadlock FP, Kent WR, Lloyd JL, et al. An evaluation of two methods for measuring fetal head and body circumference. J Ultrasound Med 1982;1:359.
12. Hill LM, Breckle R, Wolfgram KR, et al. Evaluation of three methods for estimating fetal weight. JCU 1986; 14:171.
13. SAS User's Guide: Statistics. Cary, North Carolina: SAS Institute, 1985.
14. Deter RL, Hadlock FP, Harrist RB, Carpenter RJ. Evaluation of three methods for obtaining fetal weight estimates using dynamic image ultrasound. JCU 1981;9:421.
15. Hohler CW, Kreinick CJ, Warford SHS, et al. A new method for prediction of fetal weight from ultrasound measurement of fetal abdominal circumference, thigh circumference and femur length. J Ultrasound Med 1983; 2(suppl):107.
16. McLean F, Usher R. Measurements of liveborn fetal malnutrition infants compared with similar gestation and with similar birth weight normal controls. Biol Neonate 1970; 16:215.

In utero ponderal index as a prognostic factor in the evaluation of intrauterine growth retardation

S. Yagel, D. Zacut, S. Igelstein, Z. Palti, A. Hurwitz, and B. Rosenn
Jerusalem, Israel

The in utero ponderal index has become a method of estimating fetal growth. Several methods have been used in the formulation of the in utero ponderal index. In the present study, 1040 pregnant women underwent ultrasonic examination between 24 and 41 weeks of gestation, and the in utero ponderal index was determined by dividing the estimated fetal weight by the third power of the femur length, rendering a relatively constant value of 8.345 ± 2.5 (2 SD). Fifty-one cases were defined as intrauterine growth retardation due to an estimated fetal weight lower than the tenth percentile for gestational age. The in utero ponderal index proved to be a valuable index in the prediction of fetal outcome in those cases of intrauterine growth retardation in which the in utero ponderal index was smaller than 1 SD from the average of 8.345. Fetal and neonatal well-being was clearly compromised when intrauterine growth retardation was associated with a low in utero ponderal index. (AM J OBSTET GYNECOL 1987;157:415-9.)

Key words: Intrauterine growth retardation, ponderal index, ultrasound

Ultrasonic evaluation of fetal weight is imperative for antenatal diagnosis of intrauterine growth retardation (IUGR). The most commonly accepted definition of IUGR is estimated fetal weight below the tenth percentile for gestational age.[1, 2] The accuracy of ultrasonic determination of IUGR in predicting birth weight below the tenth percentile is reported to be up to 96%.[2, 3] An ultrasonic diagnosis of a small fetus might be the result of placental insufficiency or of congenital anomalies causing growth limitation and other abnormal conditions[1] but may also represent a constitutionally small fetus. Furthermore, most methods of ultrasonic diagnosis are scaled to gestational age, while accurate gestational age may not always be accurately established. This fact alone is apt to lead to erroneous diagnosis of IUGR.

For more than 25 years, pediatricians have been implementing Rohrer's ponderal index in evaluating fetal growth impairment.[1] It has been shown to be a useful parameter in qualifying the effect of intrauterine malnutrition on the newborn infant. The ponderal index is defined as birth weight × 100/(crown-heel length),[3] and its mean value varies from 2.25 at 30 weeks of gestation to 2.55 at 38 weeks.[1] Recently, several variations of the ponderal index have been suggested to represent the fetal ponderal index.[5, 6] These indices are based on the fetal femoral length as representing the fetal length[7] and on the fetal abdominal circumference, which is known to be closely related to the fetal weight.[6] The analogous fetal ponderal indices were all found to maintain a constant value between 20 and 40 weeks of gestation, and their importance and usefulness in evaluating fetal growth in high-risk pregnancies lacking established gestational ages were emphasized.

Recently, in utero ponderal index [based on estimated fetal weight/(calculated fetal length^3)] was evaluated as a predictor of IUGR in a group of 113 patients who were delivered of their infants within 72 hours of the ultrasound examination. This method was found to have a sensitivity of 76.9%, a specificity of 82%, and a negative predictive value of 96.4%.[8]

The aim of the present study was twofold: First, we aimed to determine the distribution of a modified intrauterine fetal ponderal index, defined as:

$$\text{IUPI} = \frac{\text{EFW}}{(\text{FL})^3}$$

where EFW is estimated fetal weight given in grams and FL is fetal length given in centimeters, on the basis of a large data set (1040 ultrasonic estimations of fetal weight, performed according to the method of Shepard et al.)[9]

The second aim was to investigate prospectively the value of the in utero ponderal index in cases with estimated fetal weight below the tenth percentile during the second half of pregnancy.

Material and methods

A total of 1040 gravid women underwent an ultrasonic evaluation of pregnancy between the twenty-

From the Department of Obstetrics and Gynaecology, Hadassah University Hospital, Mount Scopus.
Received for publication October 22, 1986; revised March 30, 1987; accepted April 22, 1987.
Reprint requests: Dr. S. Yagel, Department of Anatomy, Medical Sciences Building, The University of Western Ontario, London, Ontario, Canada N6A 5C1.

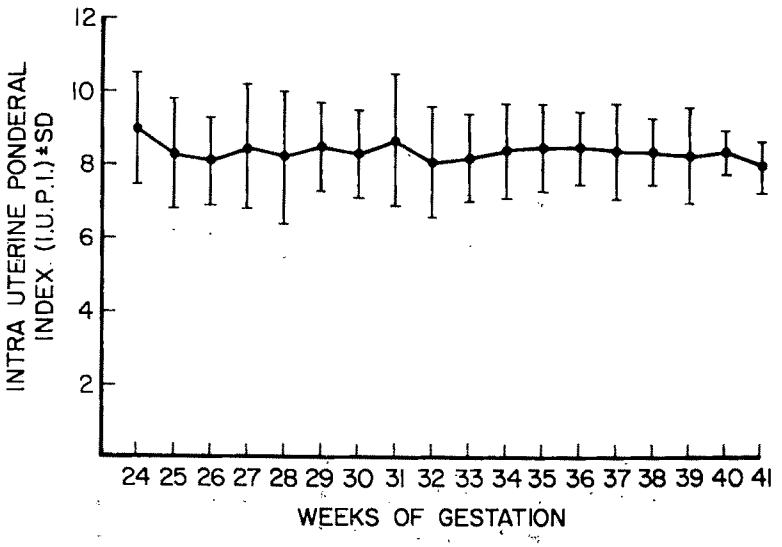


Fig. 1. Distribution of in utero ponderal index in 1040 gestations between 24 and 41 weeks.

fourth and forty-first weeks of gestation, with the use of a linear, phased array, real-time Aloka Echo camera, Model SSD-256, with a freeze frame and electronic calipers. All examinations were performed by the same operator. The 1040 patients were selected on the basis of a definitely dated last menstrual period and a history of regular menstrual cycles, with ultrasonically confirmed gestational age prior to the twenty-second week of pregnancy. Multiple gestations and those with evident malformations were excluded from the study.

Biparietal diameter measurements were obtained at the thalamic level according to the technique described by Sabbagha and Highey.[10] Femoral length measurements were carried out by use of the technique suggested by Mahoney and Hobbins.[11] Measurements were repeated until reproducible within a 1 mm range at least three times, then averaged and recorded. Abdominal circumference was calculated according to the technique described by Ott et al.[12] Fetal weight was calculated by means of the formula proposed by Shepard et al.[9] The in utero ponderal index was then calculated with the following equation:

$$\text{IUPI} = \frac{\text{EFW (in grams)}}{(\text{FL})^3 \text{ (in centimeters)}}$$

We chose the fetal length in the above formula in an attempt to be as similar as possible to the original neonatal ponderal index of Rohrer. In 51 patients (4.9%), the estimated fetal weight was found to be below the tenth percentile for gestational age, thus establishing an ultrasonic diagnosis of IUGR. The in utero ponderal index was calculated at the time of diagnosis but was withheld from the treating physicians. These pregnancies with IUGR were thoroughly followed up throughout delivery in the following manner: (1) Repeated ultrasonographic evaluation, including estimated fetal weight, placental grading, and biophysical scoring according to Manning and Morrison[13] (amniotic fluid volume, fetal tone and body movements, fetal breathing movements, and nonstress testing from the thirty-second week of pregnancy onward), was performed at intervals of 2 to 3 weeks up to the thirty-sixth week of pregnancy, and then at 5-day intervals. (2) Possible underlying maternal causes were sought, and tests were taken for markers of collagen disease, recent viral infections, anemia, diabetes mellitus, hypertensive disorders, renal disorders, etc. (3) The umbilical arterial wave pulse was recorded according to Schulman et al.,[14] in some of the patients, for determination of the systolic/diastolic ratio. (4) Examination of the neonate included Apgar scoring; blood testing for complete blood count, electrolytes, calcium, glucose, and thyroid hormone levels; Dubowitz scoring; determination of the ponderal index[4] according to Rohrer; and brain sonography for evaluation of intraventricular hemorrhage.

Results

Among the 1040 patients undergoing ultrasonic evaluation between 24 and 41 weeks of gestation, 93% had a completely normal pregnancy. The remaining 7% comprised patients with IUGR, polyhydramnios, premature rupture of the membranes, abruptio placentae, and intrauterine fetal death. Gestational age was estimated in each case, on the basis of biparietal diameter and fetal length,[15] and was found to be compatible with gestational age in 95% of the cases. In 2% of the cases, estimated age was smaller than clinical age by >2 SD, and in half of these cases, a decreased amount of amniotic fluid was observed, namely, pockets measuring

Table I. Antepartum well-being

Group	*Biophysical profile score ≤ 6**		*Intrapartum fetal distress*		*Intrauterine fetal death*		*Delivery by cesarean section*		*Systolic/diastolic ratio† >3 : 1*
	n	%	*n*	%	*n*	%	*n*	%	
Normal intrauterine ponderal index (n = 30)	5	16.6	2	6.6	0	0	3	10	0
Abnormal intrauterine ponderal index (1 SD) (n = 21)	10	47.6	5	23.8	2	9.5	7	33.3	4 of 7
p Value	<0.01		<0.05		<0.01		<0.05		

*Last biophysical profile score prior to delivery.

†Was determined in only nine patients in the normal group and seven patients in the abnormal group.

Table II. Neonatal outcome

Group	*5 min Apgar score ≤ 7*		*Intraventricular hemorrhage**		*Abnormal blood biochemistry results†*		*Small for gestational age*		*Abnormal ponderal index‡*		*Neonatal death*	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Normal intrauterine ponderal index (n = 30)	0	0	0	0	2	6.6	5	16.5	1	3.3	0	0
Abnormal intrauterine ponderal index (<1 SD) (n = 19§)	5	26.3	4	21	7	36.8	9	47.4	9	47.4	2	10.5
p Value	<0.01		<0.01		<0.01		<0.01		<0.01			

*Grade ≥ II.

†One or more abnormal result.

‡Lower than tenth percentile for gestational age.

§There were two intrauterine fetal deaths in the group of 21 patients with abnormal intrauterine ponderal indexes.

≤5 cm at the largest dimension. In 3% of the cases, estimated age was greater than clinical age by >2 SD. In 22% of these, polyhydramnios was evident, with no apparent anatomic cause.

The in utero ponderal index, calculated via the formula given, is clearly seen to maintain a relatively constant value of 8.345 during the second half of gestation, as exemplified by Fig. 1.

The second study group comprised 51 cases suspected to have IUGR as diagnosed by ultrasonic examination. Thirty of these fetuses were found to have a normal in utero ponderal index, while 21 had an abnormal in utero ponderal index that was ≥1 SD below the mean. Fetal and neonatal outcomes, together with clinical descriptions of the compromised neonates, are summarized in Tables I to III.

The group with an abnormal in utero ponderal index was found to have a higher incidence of a low biophysical score (≤6), namely, 47.6% versus 16.6% in the normal group, and a higher incidence of intrapartum fetal distress (23.8% versus 6.6%). As might be expected, there was a higher rate of delivery by cesarean section in the abnormal group (33.3% versus 10%) and of intrauterine fetal death (9.5% versus 0%). In seven patients in the abnormal group, Doppler determination of the umbilical artery systolic/diastolic ratio was performed and found to be >3 : 1 in four. None of the nine patients in the normal group undergoing the examination showed abnormal results.

A low Apgar score of ≤7 at 5 minutes post partum was more common in the abnormal group (26.3% versus 0%), as were abnormal results of blood biochemistry studies (36.8% versus 6.6%). Not one case of intraventricular hemorrhage was diagnosed in the normal group as opposed to four cases (21%) in the abnormal group. There were two neonatal deaths among the abnormal group and none in the normal group. A postpartum diagnosis of neonates that were small for gestational age was assigned in 47.4% of the abnormal in utero ponderal index group as opposed to only

Table III. Pregnancies with abnormal intrauterine ponderal index and abnormal outcome

Case No.	*Possible cause of IUGR*	*Gestational age at diagnosis*	*Intrauterine ponderal index at diagnosis*	*Biophysical score*	*Route of delivery*	*Gestational age at delivery*	*Birth weight (gm)*	*Apgar score at 5 min*	*Ponderal index at delivery**	*Neonatal or fetal outcome*
1	Pregnancy-induced hypertension	38	6.8	—	Vaginal delivery	39	1960	—	—	Intrauterine fetal death
2	Unknown	32	6.4	8	Vaginal delivery	39	2410	9	1.91 (2.30)	—
3	Unknown	35	5.6	8	Vaginal delivery	40	2360	9	2.25 (2.30)	—
4	Diabetes mellitus	33	5.6	6	Cesarean section	37	2220	6	2.05 (2.58)	Intraventricular hemorrhage, grade 2
5	Unknown	32	6.3	6	Cesarean section	39	2460	7	2.20 (2.30)	
6	Circulating anticoagulants	26	6.6	2†	Vaginal delivery	28	590	—	—	Intrauterine fetal death
7	Unknown	29	6.4	4†	Vaginal delivery	35	1550	2	1.95 (2.20)	Neonatal death; intraventricular hemorrhage, grade 3
8	Pregnancy-induced hypertension	31	4.6	4	Cesarean section	39	2400	6	2.10 (2.30)	Neonatal death; intraventricular hemorrhage, grade 4
9	Systemic lupus erythematosus	30	5.3	6	Cesarean section	34	1560	8	1.85 (2.18)	Intraventricular hemorrhage, grade 2
10	Unknown	29	6.4	6†	Cesarean section	36	1900	7	2.10 (2.23)	—
11	Unknown	28	4.7	4†	Cesarean section	30	750	6	1.90 (2.05)	Retrolental fibroplasia

*The tenth percentile for ponderal index at the given gestational age is stated in parentheses.
†The value of the biophysical profile score at <32 weeks' gestational age has not been established.

Table IV. The prognostic ability of the intrauterine ponderal index in cases of suspected IUGR

True negative	= 29/51
False negative	= 1/51
False positive	= 10/51
True positive	= 11/51
Sensitivity	= 91.6%
Specificity	= 74.3%
Positive predictive value	= 52.4%
Negative predictive value	= 96.7%
Accuracy	= 78.4%
Prevalence	= 23.5%

16.5% in the group with a normal in utero ponderal index. A low neonatal ponderal index (1 SD below normal) was predominant in the abnormal group, manifesting itself in 47.4% of the cases, while only 3.3% of the normal group had a consequently low neonatal ponderal index.

Table IV summarizes the prognostic ability of the in utero ponderal index in cases of suspected IUGR.

Comment

An ultrasound diagnosis of IUGR in the latter half of pregnancy presents the problem of consequent follow-up and treatment. Small for gestational age newborn infants are known to suffer from increased perinatal morbidity and mortality, but, on the other hand, antenatal ultrasound diagnosis of IUGR has failed to exhibit a significant correlation with a compromised fetal outcome.[16] This somewhat surprising fact may be related to several factors: (1) Ultrasound-determined estimated fetal weight is well known to have an error between 4% and 15%,[12] and even the slightest inaccuracy in weight estimation may make the difference between a diagnosis of small for gestational age and one of appropriate for gestational age. (2) Many infants, although below the tenth percentile of weight for gestational age, are nonetheless normal and healthy. (3) A misdiagnosis of IUGR is sometimes due to an erroneous gestational age.

Two facts clearly stand out in our present study: First, the in utero ponderal index maintains a constant value, a fact that is in accordance with recent reports[5, 6]; second, the in utero ponderal index clearly differentiates between two distinct groups of fetuses and may serve as a guide in evaluating the ultrasonic data and in deciding on subsequent follow-up and treatment.

Pregnancies with a diagnosis of small for gestational age with a normal in utero ponderal index had the same

outcome as that in the general population. Most (83.5%) of these infants had birth weights within normal limits, and indeed in 80% a normal estimated fetal weight was established during subsequent ultrasound examinations in the follow-up period. This fact probably represents the inaccuracy of ultrasound weight estimation combined with the rigid cutoff point at the tenth percentile. A total of 16.5% of the pregnancies were concluded with the birth of a small for gestational age but otherwise normal baby, with a normal ponderal index; these babies are constitutionally small. Only one newborn infant had evidence of malnutrition, exemplified by a low ponderal index.

On the other hand, the perinatal outcome of the group with an abnormal in utero ponderal index was devastating. Among the 21 pregnancies, there were two intrauterine fetal deaths, two neonatal deaths, and four cases of intraventricular hemorrhage. Five newborn babies had low Apgar scores (≤ 7), and nine of 19 had a ponderal index lower than the tenth percentile for gestational age. The abnormal in utero ponderal index also proved to correlate with findings indicative of placental insufficiency and of fetal compromise; four of seven patients had Doppler-measured umbilical artery systolic/diastolic ratios of $>3:1$, 10 of 21 had a decreased amniotic fluid volume, and eight had a high placental grading (Grade III) before delivery. A biophysical profile score of ≤ 6 was found in 10 of 21 pregnancies with low in utero ponderal indexes, five exhibited intrapartum fetal distress, and six were delivered by cesarean section.

The fact that the in utero ponderal index was found to maintain a constant value throughout the second half of pregnancy is in obvious discordance with the nomograms describing neonatal ponderal index according to gestational age, which show a gradual increase in the ponderal index from 30 to 38 weeks of gestation.[1] This discrepancy might be explained by the fact that the data base in designing the nomograms was most probably biased by intrauterine growth retardation in premature newborn infants with a low ponderal index. Our relatively constant in utero ponderal index was derived from ultrasound determinations in pregnancies, the majority (95%) of which were normal, and is in accordance with that of others.[5, 6]

The value of determining the in utero ponderal index in cases of suspected IUGR is summarized in Table IV. In our study group, a fetus in the third trimester with an ultrasound diagnosis of IUGR and an abnormally low in utero ponderal index had a 50% chance of being malnourished and a 30% chance of suffering some kind of severe morbidity or dying. Conversely, a normal in utero ponderal index, in the presence of IUGR in this group, was associated with a normal outcome in 96.7% of the cases.

In conclusion, although the sensitivity and the positive predictive value of the described method do not support it as a diagnostic screening procedure, the data do indicate that if IUGR is diagnosed then an abnormal in utero ponderal index places the fetus in a particularly high-risk category. However, this method may help in the follow-up of fetuses believed to have IUGR.

REFERENCES

1. Seeds JW. Impaired fetal growth: definition and clinical diagnosis. Obstet Gynecol 1984;64:303.
2. Kurjak A, Kirkinen P, Latin V. Biometric and dynamic ultrasound assessment of small-for-date infants: report of 260 cases. Obstet Gynecol 1980;56:281.
3. Ott WJ, Doyle S. Ultrasonic diagnosis of altered fetal growth by use of abnormal ultrasonic weight curve. Obstet Gynecol 1984;63:201.
4. Miller HC, Hassonein K. Diagnosis of impaired fetal growth in newborn infants. Pediatrics 1971;48:511.
5. Vintzileos AM, Neckles S, Campbell WA, et al. Three fetal ponderal indexes in normal pregnancy. Obstet Gynecol 1985;65:807.
6. Hadlock FP, Deter RL, Harrist RB, et al. A date independent predictor of intrauterine growth retardation: femur length/abdominal circumference ratio. Am J Roentgenol 1983;141:979.
7. Hadlock FP, Deter RL, Roecker E, et al. Relation of fetal femur length to neonatal crown-heel length. J Ultrasound Med 1984;3:1.
8. Vintzileos AM, Lodiero JG, Feinstein SJ, et al. Value of fetal ponderal index in predicting growth retardation. Obstet Gynecol 1986;67:584.
9. Shepard MJ, Richards WA, Berkowitz RL, et al. An evaluation of two equations for predicting fetal weight by ultrasound. Am J Obstet Gynecol 1982;142:47.
10. Sabbagha RE, Highey M. Standardization of sonar cephalometry and gestational age. Obstet Gynecol 1978;52:402.
11. Mahoney MJ, Hobbins JC. Prenatal diagnosis of chondroectodermal dysplasia (Ellison Creveld syndrome) with fetoscopy and ultrasound. N Engl J Med 1977;297:258.
12. Ott WJ, Doyle S, Flamm S. Accurate ultrasonic estimation of fetal weight. Am J Perinatol 1985;2:178.
13. Manning FA, Morrison I. Fetal biophysical profile scoring: a prospective study in 1,184 high-risk patients. Am J Obstet Gynecol 1981;140:389.
14. Schulman H, Fleischer A, Stern W, et al. Umbilical velocity wave ratios in human pregnancy. Am J Obstet Gynecol 1984;148:985.
15. Yagel S, Adoni A, Oman S, et al. A statistical examination of the accuracy of combining femoral length and biparietal diameter as an index of fetal gestational age. Br J Obstet Gynaecol 1985;92:111.
16. Bokketeig LS, Eik-Nes SH, Jacobsen G, et al. Randomized controlled trial of ultrasonographic screening in pregnancy. Lancet 1984;2:207.

Nerve entrapment after Pfannenstiel incision

William C. Sippo, M.D., Captain, MC, USA, Anne Burghardt, M.D., Major, MC, USA, and Alphonse C. Gomez, M.D., Colonel, MC, USA

El Paso, Texas

In a 16-month period, nine cases of iliohypogastric nerve entrapment were diagnosed in patients who had undergone a Pfannenstiel incision. Three patients experienced total relief with nerve blocks alone; four patients required surgical interruption of the iliohypogastric nerve to attain total relief; two patients received significant symptomatic relief with injection and desired no further treatment. The presentation, diagnosis, treatment, and prevention of entrapment of the iliohypogastric nerve as a complication of the Pfannenstiel incision are presented and discussed. (AM J OBSTET GYNECOL 1987;157:420-1.)

Key words: Iliohypogastric nerve, nerve entrapment, Pfannenstiel incision

The purpose of this article is to identify iliohypogastric nerve entrapment as a common complication of the Pfannenstiel incision and to present an approach to the diagnosis, treatment, and prevention of this complication.

Material and methods

During the 16-month period September 1, 1984, to December 31, 1985, nine patients with the complaint of lower abdominal pain, a history of a Pfannenstiel incision, and a negative workup for their symptoms were evaluated with a diagnostic ilioinguinal-iliohypogastric nerve block.

Six patients had had one procedure and three had had multiple procedures via Pfannenstiel incision.

Seven patients noted the onset of symptoms within 3 years, and two patients had symptoms more than 10 years after the last procedure.

The patients' histories were strikingly similar, being characterized initially by activity-induced, intermittent sharp pain that emanated from the incision site and radiated into the labia and/or upper inner aspect of the thigh and then over weeks to months progressed into a constant dull, burning pain within this same distribution.

The patients were placed in a supine position, with the region from the umbilicus to the upper thigh exposed on the side involved. A 3-inch, 25-gauge spinal needle was guided with a gloved hand beneath the external oblique aponeurosis at a point 1 inch medial and 1 inch inferior to the anterior superior iliac spine. With a 2:1 mixture of 0.5% bupivacaine and 1.0% lidocaine without epinephrine, 1 ml was deposited at this site. The needle was advanced toward the pubic tubercle, and on its withdrawal 3 ml of the mixture was injected with steady, even pressure. Without removal of the needle, it was redirected such that, in three or more passes, a fan-shaped deposition of anesthetic was accomplished (the last injection being perpendicular to the midline) (Fig. 1). The needle was directed beneath the rectus fascia when it was encountered.

The patients were evaluated on a weekly basis and underwent reinjection if symptoms recurred. Patients whose symptoms recurred after a third injection were considered for surgical intervention.

Results

All nine patients reported immediate relief of symptoms after injection.

Total resolution of symptoms was achieved in one patient after one injection and in two patients after a second injection; these patients remained symptom free on 6-month follow-up and required no further treatment.

Two patients experienced a significant alteration of their discomfort with the block and desired no further intervention. On follow-up these patients related occasional intermittent symptoms, which they tolerated.

The four remaining patients received only transient relief after each of the three injections and underwent surgical exploration.

The first had a palpable neuroma within the rectus sheath involving a branch of the iliohypogastric nerve. Symptoms resolved after excision of the neuroma.

The second patient had surgical interruption of the ilioinguinal nerve proximal to the area of the original incision without complete relief of symptoms. Reexploration was performed subsequently with the patient under local anesthesia, at which time the iliohypogastric nerve was identified as the source of symptoms. A

From the Department of Surgery, William Beaumont Army Medical Center.

Received for publication October 16, 1986; revised January 8, 1987; accepted February 6, 1987.

Reprint requests: Captain W. C. Sippo, MC, Department of Surgery, William Keller Army Hospital, West Point, NY 10996.

1 cm portion of the nerve was excised and the proximal and distal ends were ligated. This patient experienced complete relief.

The last two patients likewise underwent exploration while under local anesthesia and had resolution of symptoms after interruption of the iliohypogastric nerve as described above.

Comment

Our ability to relieve symptoms in all nine patients with the ilioinguinal-iliohypogastric nerve block and the results of our surgical intervention suggest that the nerve entrapment syndrome associated with the Pfannenstiel incision is due to injury of the iliohypogastric nerve.

Nerve entrapment may result from incision of a nerve with formation of a neuroma, from incorporation of a nerve by suture in the closure of the fascia, or from the tethering and constriction of a nerve in the surrounding scar that results from the normal healing process.[1]

Injury to the iliohypogastric nerve is more likely to occur either when the fascial incision extends beyond the lateral edge of the rectus sheath and into the substance of the internal oblique muscle where this nerve runs or when the corners of this fascial incision are closed with widely placed sutures, which may incorporate distal branches of the nerve in the closure.

There are two formally described methods of extension of the Pfannenstiel incision, both of which emphasize that the incision should be extended through the external oblique aponeurosis only and not involve incision of the internal oblique muscle.[2] In this way injury to the nerve may be avoided.

Closure of the Pfannenstiel incision does not require widely placed corner sutures to prevent incisional hernia. Injury to the nerve from closure is therefore easily avoidable by carefully placed sutures limited to the fascia.

The ilioinguinal-iliohypogastric nerve block is an important diagnostic tool in the assessment of patients with abdominal wall pain after Pfannenstiel incision. If the block relieves a patient's symptoms, then entrapment may be implicated and treatment initiated without more costtly and invasive workup.

As demonstrated by our results, the ilioinguinal-iliohypogastric nerve block is also an important therapeutic tool in the treatment of the nerve entrapment associated with Pfannenstiel incision.

Although the true incidence of this entity is unknown, the number of patients we identified with this syndrome in just 16 months suggests that this is a common complication of the Pfannenstiel incision that will be identified if actively sought.

In summary, entrapment of the iliohypogastric nerve is a common complication of the Pfannenstiel incision.

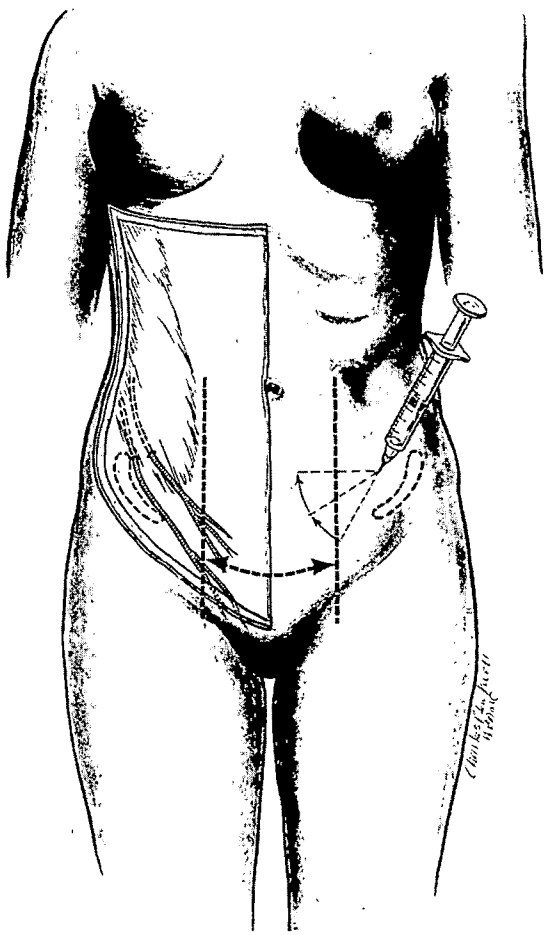


Fig. 1. The courses of the ilioinguinal nerve (lateral) and the iliohypogastric nerve (medial) are depicted on the *left*. The *vertical dashed lines* represent the borders of the rectus muscles and the *transverse dashed line* depicts the location of the Pfannenstiel incision. The pattern of injection for the nerve block is demonstrated on the *right*.

Patients present with vague, lower abdominal, burning pain in the region of the incision; this pain may radiate to the labia or upper inner aspect of the thigh. Relief of symptoms after ilioinguinal-iliohypogastric nerve block is diagnostic of the entrapment and may preclude an unnecessary costly workup. Failure of recurrent blocks to relieve the symptoms permanently suggests that a surgical interruption of the nerve is warranted.

Nerve injury may be minimized by both limiting the extension of the Pfannenstiel incision beyond the confines of the rectus sheath to the external oblique aponeurosis and avoiding the wide placement of sutures in the closure of the anterior fascia at its corners.

REFERENCES

1. Grosz CR. Iliohypogastric nerve injury. Am J Surg 1981; vol 628.
2. Shackleford RT, Zuidema GD. Surgery of the alimentary tract. 2d ed. Philadelphia: WB Saunders, 1981, vol 2: 513-4.

August 1987
Am J Obstet Gynecol

Treatment of polyhydramnios with prostaglandin synthetase inhibitor (indomethacin)

Dominique Cabrol, M.D., Robert Landesman, M.D., Joel Muller, M.D., Michele Uzan, M.D., Claude Sureau, M.D., and Brij B. Saxena, Ph.D., D.Sc.
New York, New York, and Paris, France

Polyhydramnios, which is caused by an excess of amniotic fluid, was diagnosed uniformly in eight patients by ultrasound examination and clinical symptoms, namely, premature uterine contraction, abdominal pain, and respiratory discomfort that resulted from excessive pressure on the diaphragm. Fetal anomalies incompatible with extrauterine existence were excluded by serum α-fetoprotein levels, repeated sonography, amniocentesis, and chromosomal analyses. Patients were treated with 2.2 to 3.0 mg of indomethacin/kg body weight/day. The treatment was started at an average gestational age of 24 ± 0.5 weeks and continued for 2 to 11 weeks. All patients had significant improvement with a simultaneous reduction in amniotic fluid volume, fundal height, and umbilical perimeter. All patients were delivered satisfactorily at an average of 39 weeks' gestation. The fetuses were within the normal body weight range of 2750 to 3600 gm and showed normal development during the neonatal period of 2 to 6 months. Before delivery without indomethacin, the polyhydramnios reformed. Adverse effects or intolerance to the drug was avoided by either a reduction in the dose or cessation of indomethacin therapy. Our studies suggest that indomethacin therapy is an effective way to manage pregnancy complicated by polyhydramnios. (Am J Obstet Gynecol 1987;157:422-6.)

Key words: Polyhydramnios, indomethacin, prostaglandins

Prostaglandins E_2 and F_2 are important in the regulation and maintenance of vascular tone of the blood vessels. In the lamb, calf, and goat, prostaglandins relax the pulmonary vessels and the ductus arteriosus. On the other hand, prostaglandin inhibitors such as indomethacin and salicylates increase the pulmonary vascular resistance and constrict the ductus arteriosus, which result in the elevation of the right ventricular pressure and may cause subendocardial ischemia and calcified lesions in the tricuspid valve papillary muscle and thus tricuspid insufficiency. Newborn infants may experience hypoxemia, cardiomegaly, and right to left shunting through either the foramen ovale or the ductus arteriosus. Other investigators have noted few abnormalities in newborn infants whose mothers were treated cautiously with indomethacin in lower doses.

Polyhydramnios results in poor fetal prognosis, especially during the second trimester. There is about a 30% fetal mortality rate because of premature rupture of the membranes and delivery of a fetus weighing between 500 and 700 gm. The patients are managed by repeat transabdominal amniocentesis under tocolytic treatment and withdrawal of fluid. Multiple punctures frequently result in infection or premature rupture of the membranes. The therapeutic use of prostaglandin synthetase inhibitors decreases the amniotic fluid volume and prevents premature labor.[1,2] This approach is based on the known contribution of fetal urinary flow in the production of amniotic fluid and a decreased rate of urinary flow in the premature fetus treated with indomethacin for patent ductus arteriosus.[3,4] In addition, pregnant animal models and women treated with indomethacin for premature birth developed oligohydramnios.[5,6] Therefore indomethacin was chosen to treat polyhydramnios in eight patients.

Material and methods

The patients enrolled in the study had definite clinical polyhydramnios as determined by a fundal height more than 3 cm above that at the normal gestational age (Table I) and confirmed by ultrasonography. Polyhydramnios was defined in our study by the presence of the largest pocket of amniotic fluid at a depth of 8 cm.[1,2] Polyhydramnios was associated with abdominal pain, respiratory discomfort, and premature uterine contractions. Fetal anomalies incompatible with extrauterine existence were excluded by ultrasonography. Karyotype and α-fetoprotein determinations in the aliquot of 20 ml of amniotic fluid aspirated by transab-

From the Clinique Universitaire Baudelocque and the Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, The New York Hospital–Cornell Medical Center.
Supported by the Robert Wood Johnson Jr. Charitable Trust.
Received for publication August 7, 1986; revised March 23, 1987; accepted April 20, 1987.
Reprint requests: Robert Landesman, M.D., Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, The New York Hospital–Cornell Medical Center, 1300 York Ave., New York, NY 10021.

Table I. Gestational age, fundal height, and etiology at the time of diagnosis of the polyhydramnios

Patient No.	Diagnosis of polyhydramnios		
	Gestational age (wk)	Fundal height (cm)	Etiology
1	21.5	24	None
2	24	30	Chemical diabetes
3	33	31	None
4	34	33	None
5	28.5	29	Chemical diabetes
6	28	28	Chemical diabetes
7	26	27	Chemical diabetes
8	31	32	None

dominal amniocentesis were used to exclude encephaloceles. The patients signed an informed consent form.

Eight patients, numbered 1 through 8, were treated with indomethacin for polyhydramnios. Patient No. 2 with polyhydramnios had a premature delivery at 28 weeks with neonatal death in 1981 and a late abortion at 26 weeks with a second neonatal death in 1982. Patient No. 4 had polyhydramnios but was successfully delivered of a full-term female fetus weighing 3400 gm. Four patients, namely, Nos. 2, 5, 6, and 7, had chemical diabetes, and the other four had no known maternal or fetal disease (Table II). Treatment with indomethacin began in all eight patients between 24 and 35½ weeks of gestation and at an average gestational age of 30.1 + 0.5 weeks (Table III). Patient ages ranged from 25 to 39 years, and parity was from 1 to 4, as shown in Table II. Indomethacin was administered either orally as tablets or as vaginal suppositories at a dose of 2.2 to 3.0 mg/kg body weight/day. Treatment was usually stopped between 35 and 38 weeks, and optimal care was taken to sustain the pregnancy by providing complete bed rest and if necessary by the administration of β-mimetic drugs orally for 1 week.

The effects of indomethacin therapy were evaluated daily to determine (1) a decrease in maternal symptoms and uterine contractions, (2) measurement of fundal height and umbilical circumference, and (3) weekly ultrasonographic findings to monitor fetal growth and development, as well as the approximate volume of amniotic fluid. The technique of quantitatively measuring the amniotic fluid volume consisted of measuring the depth of the largest pocket during the second half of the pregnancy, and the polyhydramnios was arbitrarily classified on the basis of the clinical impression gained while the biophysical examination of the uterine contents as described by Chamberlain et al.[7] was performed as follows: (1) >1 cm as grade 1, oligohydramnios; (2) 1 to 3 cm as grade 2, decreased fluid; (3) 3 to 8 cm as normal grade 3; and (4) >8 cm as grade 4, polyhydramnios. If under treatment severe oligohydramnios or complete interruption of fetal urinary flow developed, the dose of indomethacin was either decreased or the therapy was discontinued. Patients were evaluated for other causes of polyhydramnios such as hyperglycemia, rhesus blood grouping, irregular agglutinins, toxoplasmosis, and rubella antibodies during the course of treatment with indomethacin.

Table II. Characteristics of the eight patients (age, parity, and previous polyhydramnios)

Patient No.	Age (yr)	Parity	Previous polyhydramnios
1	25	1	—
2	26	2	2
3	33	4	—
4	27	2	1
5	32	3	—
6	39	1	—
7	30	1	—
8	29	1	—

Table III. Schedule of indomethacin treatment

Patient No.	Gestational age at time of indomethacin treatment (wk)	
	Started	Ended
1	27	35
2	24	35
3	33.6	35.5
4	34	36
5	31	35
6	32.2	35.2
7	29	34.5
8	31	38

Results

As shown in Table I, the diagnosis of polyhydramnios was confirmed between 21.5 and 34 weeks by sonogram in all eight patients. Table III shows that the longest duration that indomethacin was tolerated for was 11 weeks (patient No. 2) and the shortest duration was only 2 weeks (patient No. 4). Table IV illustrates that the deliveries were normal, spontaneous, vaginal, and at an average gestational age of 38.6 + 1.6 weeks with an Apgar score of 5 for 1 minute and 10 for 5 minutes. Patient No. 1 had a cesarean because of acute fetal distress. All eight infants weighed between 2750 and 3600 gm at birth. Careful examination during the neonatal period (2 to 6 months) revealed that all physical findings were within normal limits. There were no premature deliveries.

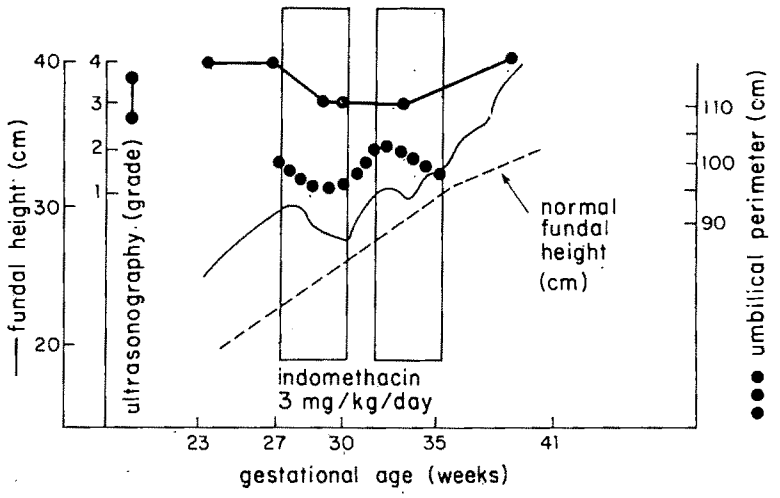


Fig. 1. Fundal height, umbilical perimeter, and qualitative measurement of amniotic fluid volume in patient No. 1 whose polyhydramnios was treated with indomethacin (3 mg/kg/day). *Dashed line* indicates normal fundal height.

Table IV. Delivery and neonatal status in the eight cases of polyhydramnios treated with indomethacin

	Delivery		*Infant*			
Patient No.	*Gestational age (wk)*	*Mode*	*Sex*	*Weight (gm)*	*Apgar score (1 min, 5 min)*	*Pediatric examination†*
1	39	Cesarean section*	M	3230	5-10	Normal
2	37	Normal delivery	F	2750	9-10	Normal
3	39.5	Normal delivery	F	3310	10-10	Normal
4	40	Normal delivery	F	3450	9-10	Normal
5	37	Normal delivery	M	2750	9-10	Normal
6	37	Normal delivery	M	2905	9-10	Normal
7	41	Normal delivery	F	3470	10-10	Normal
8	39	Normal delivery	M	3600	9-10	Normal

*Cesarean section for acute fetal distress during labor.

†Physical examination during neonatal period and at 2 or 6 months.

As shown in Fig. 1, indomethacin was administered to patient No. 1 at a dose of 3 mg/kg/body weight/day from 27 to 30 weeks and from 32 to 35 weeks in patient No. 1. After the administration of indomethacin for 10 days, there was a decrease in maternal symptoms. The uterine contractions ceased, and fundal height and umbilical circumference were decreased with normal fetal growth as confirmed by ultrasonography. The normal fundal height dropped while on indomethacin and reversed while indomethacin was withdrawn from 30 to 32 weeks. When the indomethacin was continued as long as 35 weeks, fundal heigh was maximal. The umbilical perimeter did not rise while the patient took indomethacin whereas the grade of amniotic fluid volume dropped. After cessation of indomethacin therapy, the grade of the amniotic fluid rose back to ½ cm. The patient delivered satisfactorily at 39 weeks' gestation; however, polyhydramnios was reformed. To avoid fetal distress, a cesarean was performed, and the Apgar score was 5 at 1 minute and 10 at 5 minutes. The infant weighed 3230 gm. Fig. 2 demonstrates the effects of indomethacin over a 2-week period in patient No. 5. The ultrasonographic grade of amniotic fluid volume dropped from 4 to 2 cm, and the fundal height dropped to less than the average. The umbilical perimeter was reduced from 94 to 90 cm. The patient delivered at 37 weeks' gestational age. The fetus weighed 2750 gm and had an Apgar score of 9 at 1 minute and 10 at 5 minutes. In patient No. 8 (Fig. 3), indomethacin was administered from weeks 31 to 38. The amniotic fluid volume, the umbilical perimeter, and the height of the fundus decreased. As soon as indomethacin was stopped, the amniotic fluid rapidly increased. At the time of delivery (39 weeks' gestation), the polyhydramnios had again recurred. However, this fetus was the largest of the eight fetuses (3600 gm) and had an Apgar

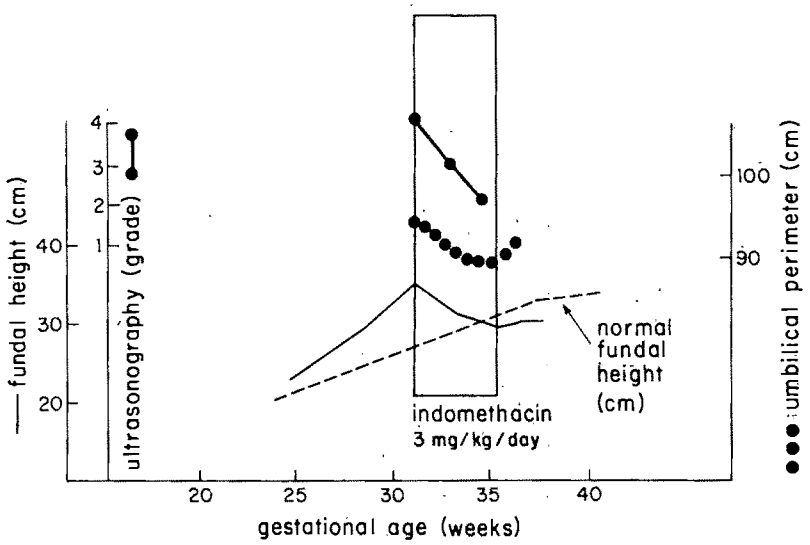


Fig. 2. Fundal height, umbilical perimeter, and qualitative measurement of amniotic fluid volume in patient No. 5 whose polyhydramnios was treated with indomethacin (3 mg/kg/day). *Dashed line* indicates normal fundal height.

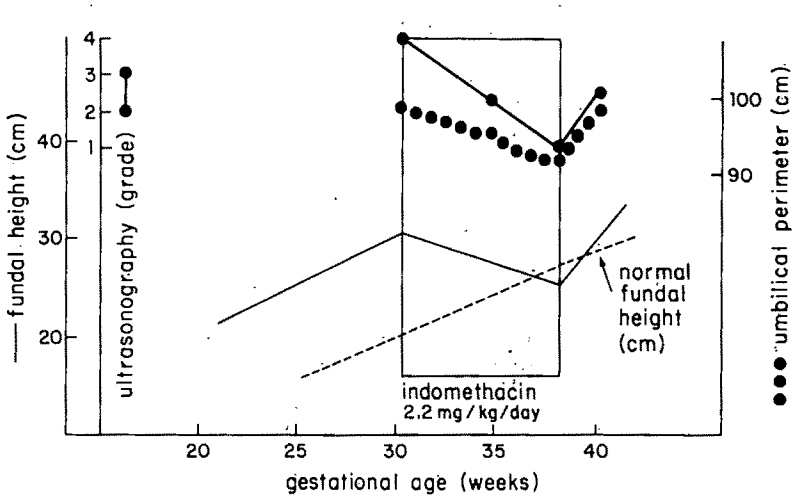


Fig. 3. Fundal height, umbilical perimeter, and qualitative measurement of amniotic fluid volume in patient No. 8 whose polyhydramnios was treated with indomethacin (2.2 mg/kg/day). *Dashed line* indicates normal fundal height.

score of 9 at 1 minute and 10 at 5 minutes. The indomethacin successfully prevented prematurity at 31 weeks and produced a mature infant at 39 weeks.

Comment

In the present study, indomethacin reduced the polyhydramnios in all eight patients. The reduction in the polyhydramnios, which was determined by measuring fundal height and umbilical perimeter, was noted by sonography after a week of therapy. The indomethacin reduced the polyhydramnios by reducing urinary flow and also minimized the amount of fluid produced by the amnion and chorion. Indomethacin was stopped during weeks 35 to 37 to prevent prematurity. We obtained improved clinical and ultrasonographic parameters in all eight patients and were able to avoid premature delivery. The fetuses were of normal size and all weighed more than 2500 gm.

The impact of prostaglandin synthetase inhibitors on amniotic fluid dynamics is not known. It is postulated that they probably decrease fetal urinary output[5, 6] or act on amniotic fluid reabsorption via the lungs.[8] Kitterman and Liggins[9] demonstrated that prostaglandin synthetase inhibitors increase fetal respiratory movements. Indomethacin could also act on fetal membranes, since these tissues, which perhaps play a role

in water transport, contain large amounts of prostaglandins. To elucidate the mechanism of action of indomethacin, more accurate quantitation of amniotic fluid volume and measurement of fetal urinary output before and during treatment as described by Campbell et al.[10] are required.

The risk of treating polyhydramnios with prostaglandin synthetase inhibitors is the generation of fetal anomalies that may contraindicate treatment with this drug. The risk may be minimized by repeated ultrasonographic examinations and frequent karyotyping and regulating the dose of the indomethacin. There was no premature closure of the ductus arteriosus. Long-term follow-up of indomethacin-treated infants has shown no residual effects on renal or cardiopulmonary function.[11] Risks could also be minimized by discontinuation of the drug at 35 weeks of gestation and postponement of delivery for at least 1 week at the end of treatment to prevent complications after delivery in patients treated with indomethacin.[12] Indomethacin-induced oligohydramnios or an interruption of fetal urinary flow, a complication described by Veersema et al.,[13] could be avoided by a decrease in the dose or discontinuation of the treatment. When the indomethacin therapy was terminated, the polyhydramnios reaccumulated, which might have initiated labor by distending the uterus and might have resulted in rupture of the membranes. Prostaglandin synthetase inhibitors, however, seem to be effective and a convenient mode of treatment of polyhydramnios. The risk to benefit ratio may depend on the clinical judgment of specific circumstances.

REFERENCES

1. Cabrol D, Uzan M, Sureau C. Traitement de l'hydramnios par l'indometacine. Rev Fr Gynecol Obstet 1983;78:10:643.
2. Cabrol D, Soubrane O, Uzan M, Poli G, Sureau C. Treatment of polyhydramnios with indomethacin [Abstract]. Arch Gynecol 1985;237(suppl):17.
3. Cifuentes RF, Olley PM, Balfe JW. Indomethacin and renal function in premature infants with persistent patent ductus arteriosus. J Pediatr 1979;95:583-7.
4. Catterton Z, Sellers B, Gray B. Inulin clearance in the premature infant receiving indomethacin. J Pediatr 1980;96:737-9.
5. Cantor B, Tyler T, Nelson RM, Stein GH. Olyigohydramnios and transient neonatal anuria. A possible association with the maternal use of prostaglandin synthetase inhibitors. J Reprod Med 1980;24:220.
6. Safar E, Maria B, Barrat J. Indometacine et fonction renale foetale. Nouv Press Med 1983;12:96.
7. Chamberlain PF, Manning FA, Morrison I, Harman CR, Lange IR. Ultrasound evaluation of amniotic fluid volume. AM J OBSTET GYNECOL 1984;150:250-4.
8. Duenhoelter JH, Pritchard JA. Fetal respiration: quantitative measurements of amniotic fluid inspired near term by human and rhesus fetuses. AM J OBSTET GYNECOL 1976;125:3-306.
9. Kitterman JA, Liggins GC. Fetal breathing movements and inhibitors of prostaglandin synthesis. Semin Perinatol 1980;4:97-100.
10. Campbell S, Wladimiroff JW, Dewhurst CJ. The antenatal measurement of fetal urine production. J Obstet Gynaecol Br Commonw 1973;80:680-6.
11. Merritt TA, White CI, Kurlinsky J, et al. Patent ductus arteriosus treated with ligation or indomethacin: a follow up study. J Pediatr 1979;95:588-94.
12. Levin DL. Effects of inhibition of prostaglandin synthesis on fetal development, oxygenation and fetal circulation. Semin Perinatol 1980;4:35-44.
13. Veersema D, De Jong PA, Van Wijck JAM. Indomethacin and the fetal renal non function syndrome. Eur J Obstet Gynec Reprod Biol 1983;16:113-21.

Human leukocyte antigen associated with endometrial carcinoma with a new classification of endometrial carcinoma based on its etiology

Tatsuya Kanuma, M.D., and Masao Igarashi, M.D., Ph.D.
Maebashi, Japan

To elucidate the relationship between endometrial carcinoma and the constitution, HLA antigen typing (A-locus, 13; B-locus, 20; C-locus, 6; DR-locus, 9) was investigated in 74 patients with endometrial carcinoma. A significant increase in two HLA antigens, Cw7 and DRw8, was demonstrated, but there was no intimate correlation between Cw7 or DRw8 and the three complications commonly associated with endometrial carcinoma—diabetes mellitus, obesity, and hypertensive disease. On the basis of these results, a new classification of endometrial carcinoma was proposed as follows: type A is positive Cw7 or DRw8 group; type B is negative for Cw7 and DRw8 and positive for the complications mentioned; type C is negative for Cw7 and DRw8, negative for the complications mentioned, and positive for DR 5; type D is a non-A, non-B, and non-C type. (Am J Obstet Gynecol 1987;157:427-32.)

Key words: Endometrial carcinoma, HLA antigen

Accumulated evidence suggests that endometrial carcinoma is often associated with specific complications[1-5] such as diabetes mellitus, obesity, hypertensive disease, and hyperestrogenism and that there are some families with a high occurrence of endometrial carcinoma.[6] One of the best ways to investigate hereditary or genetic cause of diseases is HLA antigen typing. Recently a high risk of endometrial carcinoma in Caucasian women with HLA-Aw23 was reported,[7] but unfortunately the investigation of HLA typing was limited to A, B, and C locus. This study included extensive HLA-A, B, C, and DR typing to elucidate the possibility of the existence of special types of HLA antigens in Japanese patients with endometrial carcinoma and the relationship to these complications.

Material and methods

Patients. Seventy-four patients with endometrial carcinoma that was confirmed by histologic examination were investigated on HLA typing and complications, such as diabetes mellitus, obesity, and hypertensive disease. Among these 74 patients, 53 underwent operation at the Gunma University Hospital, and the remaining 21 patients had operations at the Tohmo Cancer Center Hospital, Gunma, Japan. The age range of these patients was from 29 to 74 years, with a median of 54. Of these patients 40.5% were obese, 23.0% had diabetes mellitus, and 18.9% had hypertensive disease.

HLA typing. Twenty milliliters of heparinized blood was obtained from each patient. Lymphocytes were separated from blood samples by means of a Ficoll-Conray density gradient. The B-lymphocytes and T-lymphocytes were separated by the nylon-wool column procedure. With T-lymphocytes, HLA-A, HLA-B, and HLA-C antigens were determined by the National Institutes of Health standard two-stage microcytotoxicity method.[8] HLA antisera of 13 alleles of the A locus, 20 alleles of the B locus, and six alleles of the C locus were purchased from the Hoechst Company, Marburg, West Germany. Each lymphocyte preparation was adjusted to 1000 cells per microliter to consist of at least 90% viable lymphocytes. Lymphocytes were incubated with each of the antisera for 30 minutes at 15° to 25° C. Five microliters of rabbit complement was added to each well, and the incubation was continued for 60 minutes. Two microliters of eosin was then added, and the incubation was terminated by the addition of 10% formaldehyde. The plates were examined by phase microscopy to quantitate cell viability. A positive reaction was regarded as one that showed the presence of eosin in more than 80% of the cells, indicating cell death caused by antigen-antibody reaction. To test for 39 specific antigens, 101 alloantisera (33 for A locus, 55 for B locus, and 13 for C locus) were used. HLA-DR typing was performed with B-lymphocytes by a two-stage microcytotoxicity method that was modified by longer incubation times[9] (serum for 60 minutes at 37° C, complement for 120 minutes at 15° to 25° C). There were also 34 alloantisera used to type for nine

From the Department of Obstetrics and Gynecology, School of Medicine, Gunma University.
Received for publication June 20, 1986; revised December 18, 1986; accepted April 23, 1987.
Reprint requests: Masao Igarashi, M.D., Department of Obstetrics and Gynecology, Gunma University School of Medicine, Maebashi, Gunmaken, 371 Japan.

Table I. HLA antigen frequencies in patients with endometrial carcinoma and normal Japanese control values

	Patients with endometrial carcinoma (n = 74)		*Japanese normal control values (N = 950*)*		
HLA antigen	*n*	*%*	*n*	*%*	*Statistical significance*
A 1	0		10	1.0	NS
A 2	28	37.8	410	43.2	NS
A 3	0		10	1.1	NS
A 11	12	16.2	163	17.2	NS
Aw23	0		10	1.1	NS
Aw24	45	60.8	556	58.5	NS
A 25	0		1	0.1	NS
A 26	22	29.7	178	18.7	NS
A 28	0		10	1.1	NS
A 29	0		4	0.4	NS
A 31	7	9.5	145	15.3	NS
Aw32	0		1	0.1	NS
Aw33	8	10.8	124	13.1	NS
A X	26	35.1	47	4.9	
B 7	7	9.5	108	11.4	NS
B 8	0		2	0.2	NS
B 12	9	12.2	122	12.8	NS
B 13	0		38	4.0	NS
B 18	0		0		NS
B 27	0		8	0.8	NS
Bw35	10	13.5	133	14.0	NS
Bw38	0		4	0.4	
Bw39	4	5.4	54	5.7	NS
Bw48	1	1.4	44	4.6	NS
Bw51	16	21.6	151	15.9	NS
Bw52	15	20.3	195	20.5	NS
Bw54	10	13.5	134	14.1	NS
Bw55	3	4.1	55	5.8	NS
Bw56	3	4.1	21	2.2	NS
Bw59	3	4.1	40	4.2	NS
Bw60	12	16.2	121	12.7	NS
Bw61	10	13.5	160	16.8	NS
Bw62	9	12.2	159	16.7	NS
Bw63	0	36.5	4	0.4	
B X	27	36.5	216	22.7	
Cw1	24	32.4	305	32.1	NS
Cw2	0		7	0.7	NS
Cw3	37	50.0	442	46.5	NS
Cw4	8	10.8	88	9.3	NS
Cw5	0		2	0.2	NS
Cw7	13	17.6	20	2.1	Pc < 0.006, RR = 9.9
C X	66	89.2	698	73.5	
DR 1	4	5.4	108	12.2	NS
DR 2	36	48.6	318	36.0	NS
DR 3	8	10.8	28	3.2	NS
DR 4	24	32.4	366	41.4	NS
DR 5	7	9.5	38	4.3	NS
DRw6	1	1.4	80	9.1	NS
DR 7	2	2.7	9	1.0	NS
DRw8	23	31.1	111	12.6	Pc < 0.009, RR = 3.1
DRw9	18	24.3	203	23.0	NS
DR X	25	33.8	347	39.2	

Pc = Corrected p value; RR = relative risk; NS = not significant; A X, B X, C X, and DR X = blanks. Cw7 was significantly increased compared with control value. DRw8 was significantly increased compared with control value.

*For normal Japanese control values of DR-locus antigens N = 884.

specific antigens of Terasaki DRW TRAY, Lots 18 and 19, purchased from Veritas Company, Tokyo, Japan.

Statistical evaluation. As the control for HLA frequency, Japanese normal control values for HLA frequency, which were presented at the Eighth International Histocompatibility Workshop,[10] were used.

The results of HLA typing were assessed by two statistical approaches. One was the corrected p value and the other was the relative risk. The corrected p value

Table II. HLA typing data on each patient with endometrial carcinoma

No.	*Name*	*Age (yr)*	*A Locus*	*B Locus*	*C Locus*	*DR Locus*
1	Y. S.	72	A 2, Aw24	Bw52, Bw54	Cw 3, C X	DRw9, DR X
2	M. K.	74	A 11, A 26	B 15, Bw51	Cw 4, C X	DR 2, DR X
3	Y. S.	56	A 2, Aw24	Bw60, B X	C X , C X	DR 4, DRw8
4	R. N.	61	Aw24, Aw33	Bw60, B X	Cw 1, Cw 3	DRw8, DRw9
5	F. N.	47	A 2, A 26	Bw54, B X	Cw 1, C X	DR 3, DR 5
6	M. S.	44	A 2, Aw24	Bw52, B X	Cw 1, C X	DR 2, DR X
7	A. Y.	60	Aw24, A X	Bw52, Bw60	Cw 3, C X	DR 2, DR X
8	Y. T.	51	Aw24, A 26	Bw52, B X	Cw 3, C X	DR 2, DR X
9	H. N.	52	Aw24, A X	Bw51, B X	Cw 1, Cw 3	DR 4, DRw8
10	T. O.	56	Aw24, A 26	Bw54, B X	Cw 1, C X	DR 2, DR 4
11	S. K.	64	A 2, Aw24	Bw35, B X	Cw 3, C X	DR 2, DR 4
12	N. S.	36	Aw24, A X	Bw52, B X	C X , C X	DR 2, DRw9
13	K. U.	51	A X , A X	Bw61, B X	Cw 3, C X	DR 3, DR X
14	M. T.	67	A 26, A X	Bw61, B X	Cw 3, C X	DR 2, DRw8
15	N. Y.	45	Aw24, A X	B 12, Bw52	C X , C X	DR 5, DR X
16	Y. K.	62	Aw24, Aw33	Bw52, B X	Cw 1, Cw 3	DR 3, DR 5
17	M. T.	52	A 26, A 31	B 15, Bw60	Cw 3, C X	DRw8, DR X
18	T. K.	31	Aw24, A X	Bw51, B X	C X , C X	DR 2, DR X
19	Y. M.	58	Aw24, A X	Bw52, B X	Cw 7, C X	DR 4, DR X
20	T. H.	52	Aw24, Aw33	Bw54, B X	CW 1, Cw 3	DR 2, DR X
21	K. A.	63	A 11, Aw24	Bw51, Bw59	C X , C X	DR 2, DRw9
22	N. U.	55	Aw24, A X	Bw52, Bw60	C X , C X	DR 2, DR X
23	G. K.	55	A 2, A X	B 12, B X	Cw 1, Cw 3	DR 2, DRw8
24	W. A.	50	A X , A X	B 15, B X	Cw 1, C X	DR 2, DR 5
25	H. A.	69	A 2, Aw24	B 12, Bw39	Cw 3, Cw 7	DR 4, DR X
26	A. S.	56	A 2, A X	Bw35, Bw56	Cw 3, Cw 7	DR 4, DR X
27	F. N.	51	A 26, A X	B 12, Bw59	Cw 1, Cw 3	DR 4, DR X
28	Y. Y.	62	A 2, Aw24	Bw51, B X	Cw 1, C X	DR 2, DR 5
29	S. Y.	45	Aw24, A 26	Bw51, Bw55	Cw 1, Cw 3	DR 2, DR 3
30	N. H.	58	A 31, A X	B 12, Bw51	C X , C X	DRw8, DR X
31	T. O.	53	Aw24, A X	B 7, B 12	C X , C X	DR 2, DRw8
32	H. O.	66	A 2, A X	B 7, Bw51	C X , C X	DR 2, DRw8
33	K. S.	63	A 11, Aw24	Bw35, Bw54	Cw 1, Cw 3	DR 2, DR X
34	M. I.	54	Aw24, Aw33	Bw35, B X	C X , C X	DR 2, DR 3
35	T. T.	54	A 2, Aw24	Bw60, B X	Cw 1, Cw 3	DR 4, DRw9
36	K. K.	61	A 26, A 31	Bw51, B X	Cw 3, C X	DR 2, DRw9

Continued

(Pc) was obtained for each couple of particular antigens by means of the following equation: Pc = uncorrected p value × number of antigens examined for each locus. The relative risk (RR) for endometrial carcinoma to develop in a patient with a particular HLA antigen was calculated with the following equation: RR = A/a × b/B, where A is the numer of patients with the particular antigen, a is the number of patients without the antigen, B is the number of controls with the antigen, and b is the number of controls without the antigen.

Results

Incidence of HLA in endometrial carcinoma. As shown in Table I, six kinds of antigens, A2, A11, Aw24, A26, Aw31, and Aw33, in the HLA-A locus were found in cases of endometrial carcinoma. For the B locus, the 14 kinds of antigens shown in Table I were detected, but the incidence of any antigens in the A and B loci was not significantly different in endometrial carcinoma when compared with the Japanese control values. For the C locus, four kinds of antigens, Cw1, Cw3, Cw4, and Cw7, were detected (Table I). The incidence of Cw7 in endometrial carcinoma was 17.6%, significantly higher than in the control group (2.1%). The Pc was <0.006 and the RR was 9.9. The incidence of the other three antigens (besides Cw7) was not significantly different in endometrial carcinoma from that in the control group. For the DR locus, the nine kinds of antigens shown in Table I, DR1, DR2, DR3, DR4, DR5, DRw6, DR7, DRw8, and DRw9, were detected. Among these antigens, only DRw8 showed a significant increase (31.1%) in endometrial carcinoma, while it remained 12.6% in the control. The Pc was <0.009 and the RR was 3.1. All of the HLA typing data on each patient are presented in Table II.

Relationship between Cw7 or DRw8 and associated complications in endometrial carcinoma. As stated, it is well known that patients with endometrial carcinoma frequently have diabetes mellitus, hypertensive disease, or are obese. We decided therefore to investigate the relationship between the finding of Cw7 or DRw8 and the three frequent complications. Table III shows no correlation between the two factors.

Table II—Cont'd.

No.	Name	Age (yr)	A Locus	B Locus	C Locus	DR Locus
37	H. K.	61	A 26, A X	B 12, Bw35	Cw 3, C X	DRw9, DR X
38	W. O.	54	A 26, A X	Bw62, B X	Cw 3, C X	DR 2, DR 3
39	K. F.	73	A 2, Aw24	B X , B X	Cw 1, Cw 3	DR 4, DRw8
40	T. F.	50	A 11, Aw24	B 7, Bw39	Cw 7, C X	DR 1, DR X
41	S. Y.	60	A 2, Aw24	Bw39, Bw60	Cw 7, C X	DR 2, DRw8
42	T. S.	48	A 2, A X	Bw51, B X	Cw 1, C X	DRw8, DRw9
43	K. O.	58	A 2, A 11	Bw35, Bw62	Cw 3, Cw 4	DR 2, DR 4
44	H. A.	56	A 2, Aw24	Bw35, Bw56	Cw 3, Cw 4	DR 4, DRw9
45	I. T.	54	A 2, A X	Bw51, B X	Cw 1, Cw 7	DR 3, DRw8
46	S. K.	45	A 26, Aw33	B 7, B 12	Cw 7, C X	DR 1, DR X
47	A. C.	31	A 2, Aw24	Bw35, Bw61	Cw 3, Cw 7	DRw6, DR X
48	F. Y.	55	Aw24, A 26	Bw35, Bw61	Cw 3, C X	DR 4, DRw9
49	N. O.	45	Aw24, A 26	Bw54, Bw61	Cw 1, Cw 3	DR 7, DRw8
50	N. T.	46	A 26, A 31	Bw56, Bw61	Cw 3, C X	DRw9, DR X
51	K. N.	36	Aw33, A X	Bw39, Bw60	Cw 7, C X	DR 2, DRw9
52	U. O.	60	Aw24, Aw33	B 7, Bw61	Cw 7, C X	DR 1, DRw8
53	T. S.	51	Aw24, A 26	Bw35, Bw62	Cw 3, Cw 4	DR 4, DRw9
54	F. U.	54	A 2, A 31	Bw52, B X	C X , C X	DR 2, DR 4
55	I. N.	53	Aw24, A 26	Bw52, B X	C X , C X	DR 2, DRw8
56	K. M.	50	A 2, A 11	B X , B X	Cw 1, Cw 7	DR 4, DRw8
57	Y. M.	59	A 2, Aw24	Bw52, B X	Cw 3, C X	DR 2, DRw8
58	S. I.	55	Aw24, A X	B 7, Bw60	Cw 3, C X	DR 1, DR 4
59	M. M.	64	A 2, A 26	Bw51, Bw60	C X , C X	DR 2, DRw9
60	Y. T.	61	Aw24, A 26	Bw59, Bw61	C X , C X	DR 4, DR X
61	T. O.	68	Aw24, A X	Bw52, Bw62	Cw 3, C X	DR 2, DRw9
62	C. T.	57	A 11, A 31	Bw61, Bw62	Cw 4, C X	DR 4, DR X
63	U. K.	43	A 11, Aw33	B 12, Bw62	Cw 4, C X	DR 4, DRw8
64	Y. K.	56	A 2, A 26	Bw55, Bw61	Cw 1, Cw 7	DR 4, DRw8
65	K. O.	37	A 11, Aw24	Bw54, Bw62	Cw 4, Cw X	DR 4, DRw9
66	K. O.	63	A 2, A 11	Bw54, Bw55	Cw 1, C X	DR 4, DRw9
67	K. K.	52	Aw24, A X	Bw51, Bw52	C X , C X	DR 2, DR X
68	M. T.	49	A 11, Aw24	Bw51, Bw54	Cw 1, C X	DR 4, DRw9
69	T. S.	29	A 2, Aw24	B 7, BW48	Cw 7, C X	DR 2, DRw8
70	K. S.	58	A 2, Aw24	Bw54, Bw60	Cw 3, C X	DR 2, DR 5
71	M. M.	55	A 2, Aw24	Bw51, Bw52	Cw 3, C X	DR 2, DRw8
72	C. E.	69	A 26, A 31	Bw60, Bw62	Cw 1, Cw 3	DR 2, DR 7
73	T. T.	56	A 11, Aw24	Bw51, B X	Cw 3, Cw 4	DR 2, DR 3
74	Y. F.	52	A 2, A 26	B 15, Bw62	CW 1, CW 3	DR 5, DRw8

Table III. Correlation between positive findings of Cw7 or DRw8 and three associated complications in endometrial carcinoma

Presence or absence of complications	Positive for Cw7 or DRw8	Negative for Cw7 or DRw8	Total
Present	14	28	42
Absent	16	16	32
Total	30	44	74

The χ^2 value was calculated from this 2 × 2 table. Result was not significant ($\chi^2 = 2.093$).

Table IV. Haplotype frequencies in patients with endometrial carcinoma

Haplotype	Patients with carcinoma	Controls
Aw24-DRw8	12/74 (16.2%)	
HF	0.0370	0.0168
D	−0.0265	−0.0056
A 2-DRw8	13/74 (17.6%)	
HF	0.0789	0.0205
D	0.0430	0.0050
A 2-Cw7	8/74 (10.8%)	
HF	0.0540	0.0007
D	0.0285	−0.0017

HF = Haplotype frequency; D = delta value.

Incidence of haplotypes of HLA. As shown in Table IV, the incidence of some haplotypes of HLA in the endometrial carcinoma group and the control group[11] was compared. The haplotype frequencies of Aw24-DRw8, A2-DRw8, and A2-Cw7 were 16.2%, 17.6%, and 10.8%, respectively. The delta values show negative disequilibrium between Aw24 and DRw8 and positive disequilibrium between A2 and DRw8 and also between A2 and Cw7. These haplotype values showed a stronger linkage disequilibrium than the control values.

A new classification of endometrial carcinoma based on HLA and associated complications. According to the results obtained, patients with endometrial carcinoma can be classified into four groups on the basis of HLA typing. The first type, group A, is defined as

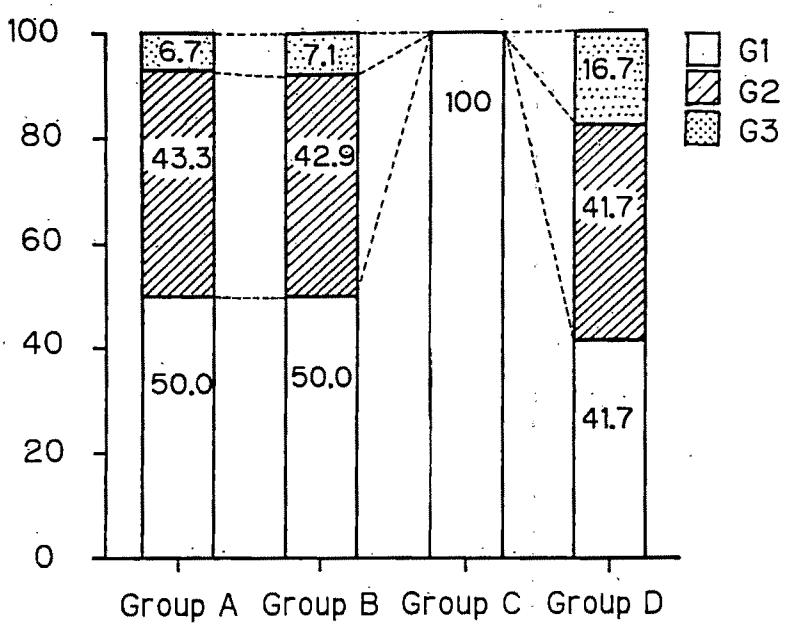


Fig. 1. Histologic differentiation of endometrial carcinoma in the four groups. G1 = Well-differentiated adenocarcinoma. G2 = Moderately differentiated adenocarcinoma. G3 = Poorly differentiated adenocarcinoma.

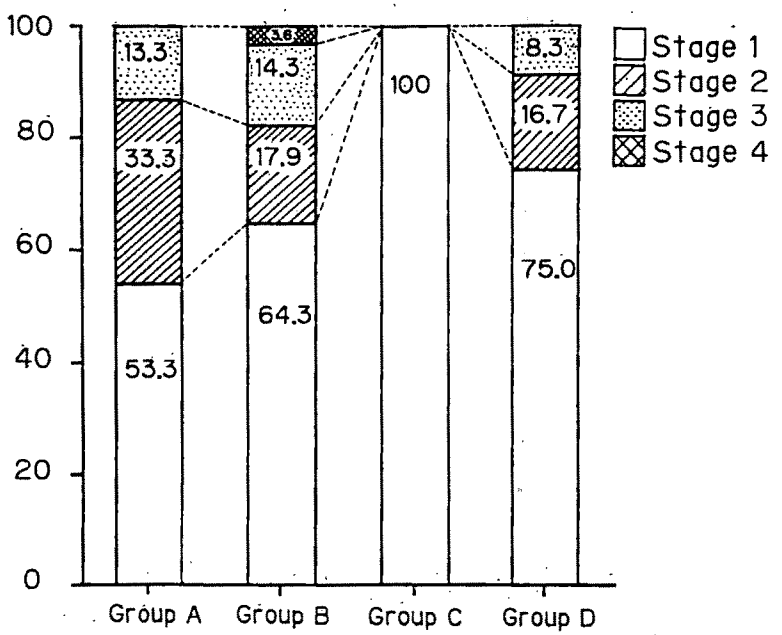


Fig. 2. Clinical stages of four groups of patients with endometrial carcinoma.

Table V. New classification of patients with endometrial carcinoma

Group	*Cw7 and/or DRw8*	*Associated complications (%)*	*DR5 (%)*	*No. of patients (n = 74)*	*Incidence (%)*
A	100	±	3.3	30	40.5
B	0	100	7.1	28	37.8
C	0	0	100	4	5.4
D	0	0	0	12	16.2

women with Cw7 and/or DRw8. As shown in Table V, 30 patients (40.5%) in the 74 cases of endometrial carcinoma belong to this group. The age range of this group was 29 to 73 years, with a median of 53. The endometrial histologic evaluation showed 50.0% G1, 43.3% G2, and 6.7% G3 (see Fig. 1). Clinical staging was as follows: Stage I, 53.3%; Stage II, 33.3%; Stage III, 13.3%; and Stage IV, 0% (Fig. 2).

Type B endometrial carcinoma is often associated with complications such as diabetes mellitus, obesity, or hypertensive disease, but not with Cw7 and DRw8 antigens. The HLA that was characteristic of this type was a high frequency of Cw3 (67.9%) or DR3 (17.9%). Twenty-eight patients (37.8%) of the endometrial carcinoma cases belong to this group (Table V). The age range was from 36 to 74 years, with a median of 57. The histologic types were 50.0% G1, 42.9% G2, and 7.1% G3 (Fig. 1). Clinical staging was 64.3% in Stage I, 17.9% in Stage II, 14.3% in Stage III, and 3.6% in Stage IV (Fig. 2).

Type C endometrial carcinoma is negative for Cw7 and DRw8, with no specific complications and positive HLA-DR5. The incidence of type C was 5.4% in 74 patients with endometrial carcinoma (Table V). The age range was from 45 to 62 years, with a median of 51. Histologic type in this group was 100% G1 (Fig. 1). Clinical staging was 100% Stage I (Fig. 2).

Type D endometrial carcinoma is defined as the non-A, non-B, and non-C type, that is, negative for Cw7, DR5, DRw8 and no particular complications. Twelve (16.2%) patients with endometrial carcinoma belong to this type (Table V). The age range was 31 to 72 years, with a median of 51. Histologic evaluation of this type showed 41.7% G1, 41.7% G2, and 16.7% G3 (Fig. 1). Clinical staging was as follows: Stage I, 75.0%; Stage II, 16.7%; Stage III, 8.3%; and Stage IV, 0% (Fig. 2).

Comment

In 1974 Simons et al.[12] reported that HLA-Bw46 had a substantial association with nasopharyngeal carcinoma; then the correlation between the HLA system and neoplastic diseases was reconsidered. Recently in the gynecologic field Sniecinski et al.[7] reported a significantly increased frequency of Aw31 in 21.0% of 38 patients with cervical carcinoma and of Aw23 in 17.6% of 17 patients with endometrial carcinoma. However, their report was based on only 17 cases of endometrial carcinoma, and there was a lack of investigation of the HLA-DR locus.

In this extensive study of the correlation between HLA antigens and endometrial carcinoma, including the examination of HLA-DR locus antigens, it was clearly demonstrated that the frequency of Cw7 and DRw8 was significantly increased in Japanese patients with endometrial carcinoma, while no associated antigens were detected in the HLA-A locus, including Aw23, and the B locus. These results, which demonstrated that no association was observed between the HLA-A and HLA-B loci and endometrial carcinoma, coincided with the reports by Takasugi et al.[13] and Terasaki et al.[14]

Since the correlation between Cw7 or DRw8 and the three complications was not demonstrated (Table I), types HLA-Cw7 and HLA-DRw8 seem to be new, constitutional, previously unreported risk factors involved in endometrial carcinogenesis.

A new classification of endometrial carcinoma based on both the HLA typing and the three associated complications was proposed according to the data obtained here.

We thank Dr. Kazuo Miyanaga, M.D., Department of Psychiatry, Gunma University School of Medicine, for his kind guidance with the HLA research methods.

REFERENCES

1. Corscaden JA, Gusberg SB. The background of cancer of the corpus. Am J Obstet Gynecol 1947;53:419.
2. Speert H. Carcinoma of the endometrium in young women. Surg Gynecol Obstet 1949;88:332.
3. Wynder EL, Escher GC, Mantel N. An epidemiologic investigation of cancer of the endometrium. Cancer 1966;19:489.
4. Hausknecht RU, Gusberg SB. Estrogen metabolism in patients at high risk for endometrial carcinoma. Am J Obstet Gynecol 1969;105:1161.
5. MacMahon B. Risk factors for endometrial cancer. Gynecol Oncol 1974;2:122.
6. Albert S, Child M. Familial cancer in the general population. Cancer 1974;34:444.
7. Sniecinski I, Haley J, Morgan JB, Nagell J. Histocompatibility-antigen distribution in patients with cervical and endometrial carcinomas. Gynecol Oncol 1981;11:68.
8. Terasaki PI, Bernoco D, Park MS, Ozturk G, Iwaki Y. Microdroplet testing for HLA-A, -B, -C and -D antigens. Am J Clin Pathol 1978;69:103.
9. Danilovs JA, Ayoub G, Terasaki PI. B lymphocyte isolation by thrombin-nylon wool. In: Terasaki PI, ed. Histocompatibility testing 1980. Los Angeles: UCLA Tissue Typing Laboratory, 1980:287-8.
10. Baur MP, Danilovs JA. Population analysis of HLA-A, B, C, DR and other genetic markers. In: Terasaki PI, ed. Histocompatibility testing 1980. Los Angeles: UCLA Tissue Typing Laboratory, 1980:958-60.
11. Baur MP, Danilovs JA. Reference table for two and three locus haplotype frequencies of HLA-A, B, C, DR, BF and GLO. In: Terasaki PI, ed. Histocompatibility testing 1980. Los Angeles: UCLA Tissue Typing Laboratory, 1980:994-1210.
12. Simons MJ, Nee GB, Day NE, Morris PJ, Shaumuaratnum K, de-The GB. Immunogenetic aspects of nasopharyngeal carcinoma. I. Differences in HL-A antigen profiles between patients and control groups. Int J Cancer 1974;13:122.
13. Takasugi M, Terasaki PI, Henderson B, Mickey MR, Menck H, Thompson RW. HL-A antigens in solid tumors. Cancer Res 1973;33:648.
14. Terasaki PI, Perdue ST, Mickey MR. HLA frequencies in cancer: a second study. In: Mulvihill JJ, Miller RW, Fraumeni JF Jr, eds. Genetics of human cancer. New York: Raven Press, 1977:321.

An evaluation of squamous cell carcinoma antigen in patients with cervical squamous cell carcinoma

Elizabeth K. Senekjian, M.D.,* J. Maitland Young, Ph.D. Patricia A. Weiser, R.N., Cheryl Eck Spencer, M.S., Susan E. Magic, M.A., and Arthur L. Herbst, M.D.

Chicago, Illinois

In a prospective study, serum concentrations of squamous cell carcinoma antigen, a subfraction of tumor antigen (TA-4), were determined by radioimmunoassay from healthy donors, pregnant women, and subjects with various benign and malignant gynecologic diseases. Ninety-six percent of 99 healthy persons including all 52 female controls, the 15 pregnant patients, and all 23 subjects with benign gynecologic tumors, had squamous cell carcinoma antigen levels <2.0 ng/ml. Seven of 51 (14%) patients with cervical intraepithelial neoplasia and 16 of 24 (67%) patients with cervical squamous cell carcinoma had squamous cell carcinoma antigen levels >2.0 ng/ml. Declining and rising levels of squamous cell carcinoma antigen, which were determined sequentially in nine cases of cervical carcinoma that were associated with elevated pretreatment levels of squamous cell carcinoma antigen, correlated with regression and progression of the disease. Serial serum levels of squamous cell carcinoma antigen provide a noninvasive means of monitoring the effects of individual therapy in patients with cervical squamous cell carcinoma. (Am J Obstet Gynecol 1987;157:433-9.)

Key words: Squamous cell carcinoma antigen, cervical squamous cell carcinoma, tumor antigen (TA-4)

Tumor antigen (TA-4), a glycoprotein with a molecular weight of approximately 48 kilodaltons,[1] was isolated by Kato and Torigoe[2] from human cervical squamous cell carcinoma tissue. Elevated TA-4 levels, which were determined by radioimmunoassay, have been found in 55% of patients with cervical squamous cell carcinoma.[1] Furthermore, fluctuations in TA-4 values have been found to correspond with tumor progression and regression.[3]

Squamous cell carcinoma antigen, one of 14 subfractions of TA-4, has been purified from cervical squamous cell carcinoma metastatic to the liver by a modification of the method described by Kato and Torigoe.[2] Maruo et al.[4] recently reported significant elevations of antigen levels in Japanese patients with advanced cervical squamous cell carcinoma and normalization of levels after radical surgery or radiotherapy.

The management of patients with cervical carcinoma is hindered by limitations in clinical staging, accurate assessment of response to therapy, and early detection of tumor recurrence. A reliable, noninvasive method that overcomes these difficulties could allow for more timely alternative or additional therapy.

In this study squamous cell carcinoma antigen values were determined in the sera of control subjects, pregnant women, and persons with benign, premalignant, and malignant gynecologic tumors. Sequential levels of squamous cell carcinoma antigen were compared with the clinical course during therapy in a group of patients with cervical carcinoma. Similar studies were performed with CA 125 and carcinoembryonic antigen. The clinical use of the three markers in patients with cervical carcinoma was evaluated.

Material and methods

Sera were obtained from 99 apparently healthy persons, 52 women and 47 men, who ranged in age from 22 to 56 years (median, 29 years) and 15 pregnant women. Pretreatment serum samples were collected from 23 patients with benign gynecologic diseases, including paraovarian cyst (one), mucinous cystadenoma (one), Brenner tumor (one), pelvic inflammatory disease (four), endometrial hyperplasia (four), uterine leiomyomata (seven), and endometriosis (eight). Specimens were obtained prospectively from 51 patients with cervical intraepithelial neoplasia, 30 with cervical carcinoma, 11 with malignancies of the uterine corpus, four with ovarian adenocarcinoma, and eight with vaginal or vulvar squamous cell carcinoma. The serum specimens were stored at −20° C until assayed for squamous cell carcinoma antigen. Review of operative re-

From the Department of Obstetrics and Gynecology, Chicago Lying-In Hospital, University of Chicago, Pritzker School of Medicine, and Abbott Laboratories.

Received for publication December 9, 1986; revised April 16, 1987; accepted April 27, 1987.

Reprint requests: Elizabeth K. Senekjian, M.D., Department of Obstetrics and Gynecology, Chicago Lying-In Hospital, 5841 S. Maryland Ave., Box 446, Chicago, IL 60637.

**Dr. Senekjian was supported in part by the American Cancer Society Clinical Career Development Award No. 85-38.*

Table I. Pretreatment detection of serum squamous cell carcinoma antigen related to stage of cervical squamous cell carcinoma

Disease stage	*Total No. cases*	*SCC level >2.0 ng/ml*	*SCC range (ng/ml)*
Stage			
I	8	4	0.3-5.4
II	7	6	1.7-5.7
III	4	3	0.0-27.7
Recurrence	5	3	1.7-265.4
Total	24	16	0.0-265.4

SCC = Squamous cell carcinoma antigen.

Table II. Combination assay of squamous cell carcinoma antigen, carcinoembryonic antigen, and CA 125 in 30 patients with cervical carcinoma

	SCC + (%)	*SCC − (%)*
CEA +		
CA 125 +	10.0	3.3
CA 125 −	3.3	10.0
CEA −		
CA 125 +	6.7	0.0
CA 125 −	36.7	30.0

SCC = squamous cell carcinoma antigen; CEA = carcinoembryonic antigen.

ports, pathology reports, and examination of biopsy and/or surgical specimens confirmed diagnoses.

Pretreatment sera were available from 24 cases of newly diagnosed and six cases of recurrent cervical carcinoma. The median age of patients with cervical carcinoma was 45 years. Histologic examination revealed that 24 lesions were squamous cell carcinomas and six were adenosquamous carcinomas. Stage was assigned according to the classification of the International Federation of Gynecology and Obstetrics. Of the 24 newly diagnosed cases, 10 patients had Stage I disease, nine had Stage II, and five had Stage III. Multiple serum samples were collected from 16 patients with cervical carcinoma; 13 had cervical squamous cell carcinoma and three had cervical adenosquamous carcinoma. Tumor regression, stability, or progression was determined by changes in the dimensions of palpable masses or lesions detected by computed tomography or chest roentgenography. Tumor regression was defined as a 50% or greater reduction in the sum of the products of the two greatest perpendicular dimensions of each measurable lesion. Tumor progression was defined as a 50% or greater increase in the result of the calculation described above or the appearance of any new lesion. Stable disease was defined as tumor behavior that did not meet either of these criteria.

Serum determinations of squamous cell carcinoma antigen were performed in duplicate by means of the radioimmunoassay kit (Abbott SCC-RIA) available through Abbott Laboratories (North Chicago, Ill.). Briefly, assay tubes containing 100 μl of sample serum or standard squamous cell carcinoma antigen solution, 200 μl of iodine 125–labeled squamous cell carcinoma antigen, and 100 μl of anti–squamous cell carcinoma antigen serum were incubated at room temperature for 20 to 30 hours. Subsequently, 500 μl of second antibody (goat antirabbit γ-globulin) solution was added. The tubes were incubated at room temperature for 10 minutes and then centrifuged at 3000 rpm for 20 minutes at 4° C. The radioactivity in the pellet was counted.

Pretreatment serum values of CA 125 and carcinoembryonic antigen were determined for each of the 30 patients with cervical carcinoma. CA 125 levels were determined by means of the Abbott CA 125 radioimmunoassay procedure. This method uses OC125 monoclonal antibodies in a solid-phase immunoradiometric assay to measure the CA 125 antigen. A CA 125 value of 35 U/ml was considered the upper limit of the normal range. The carcinoembryonic antigen concentrations were determined with the Abbott CEA–enzyme immunoassay monoclonal kit. Carcinoembryonic antigen values greater than 3.0 and 5.0 ng/ml were considered abnormal in nonsmokers and smokers, respectively. The proportions of concentrations of pretreatment serum squamous cell carcinoma antigen, CA 125, and carcinoembryonic antigen above the normal range in patients with cervical carcinoma were compared by use of the z statistic for comparison of proportions in paired samples.[5]

Results

In 99 normal persons squamous cell carcinoma antigen levels ranged from <1.0 ng/ml to 3.2 ng/ml. Ninety-six percent of these healthy blood donors had squamous cell carcinoma antigen values <1.99 ng/ml, 97% had levels <2.04 ng/ml, and 98% had values ≤2.40 ng/ml. The mean squamous cell carcinoma antigen concentration (1.24 ng/ml) plus two standard deviations (0.82 ng/ml) was computed to be 2.06 ng/ml. Therefore, when both values (2.04 and 2.06 ng/ml) were taken into consideration, a serum squamous cell carcinoma antigen level of 2.0 ng/ml was designated to be the upper limit of the normal range. All 52 normal female subjects had squamous cell carcinoma antigen levels <2.0 ng/ml.

None of the 23 patients with benign disorders of the female genital tract had elevated serum squamous cell carcinoma antigen levels (range, 0 to 1.9 ng/ml). All 15 pregnant patients had serum squamous cell carcinoma

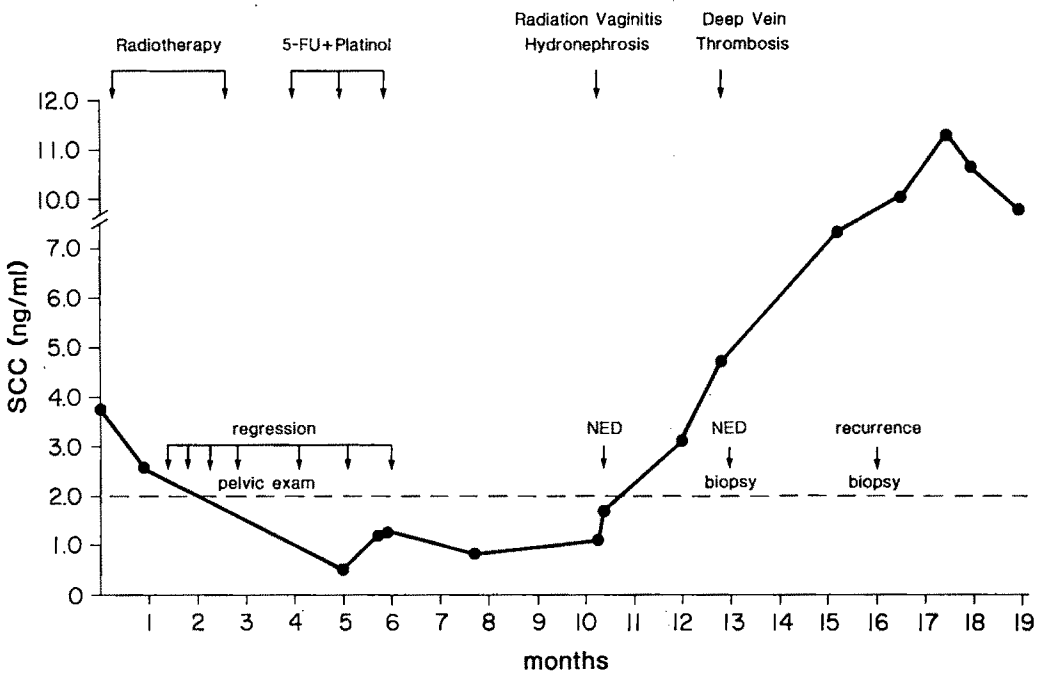


Fig. 1. Serial squamous cell carcinoma antigen *(SCC)* levels, response to therapy, and recurrence in a 30-year-old patient with a Stage IIA cervical squamous cell carcinoma. The *dotted line* denotes the upper limit for normal values of squamous cell carcinoma antigen, 2.0 ng/ml. *5FU* = 5-fluorouracil; *NED* = no evidence of disease.

antigen levels <2.0 ng/ml (range, 0 to 1.7 ng/ml). Elevated squamous cell carcinoma antigen levels were detected in seven of 51 patients with cervical intraepithelial neoplasia I, II, or III (range, 2.9 to 9.1 ng/ml). Six of the 23 patients with newly diagnosed gynecologic malignancies other than cervical carcinoma had elevated squamous cell carcinoma antigen levels (range, 2.1 to 66.6 ng/ml). Four of these latter six patients had squamous cell carcinomas of the vagina or vulva.

Among the 30 patients with newly diagnosed or recurrent cervical carcinoma, pretreatment squamous cell carcinoma antigen levels were ≤265.4 ng/ml. Seventeen (57%) of the 30 patients had elevated serum squamous cell carcinoma antigen levels. Only one of six patients with a cervical adenosquamous carcinoma had a squamous cell carcinoma antigen level in excess of 2.0 ng/ml; 16 (67%) of the 24 patients with cervical squamous cell carcinoma had elevated squamous cell carcinoma antigen levels. The distribution of serum squamous cell carcinoma antigen values by the stage of cervical squamous cell carcinoma is shown in Table I.

Of the eight cases of cervical squamous cell carcinoma with pretreatment squamous cell carcinoma antigen values <2.0 ng/ml, six had a small tumor burden. The diagnosis of Stage IB occult disease was made by cervical conization in three patients. One patient with Stage IB disease had a 2 cm cervical lesion and microscopic metastases in two pelvic nodes. In a patient with a Stage IIIB squamous carcinoma, the 2 cm cervical tumor was associated with a 0.5 cm metastasis involving the urethral meatus. One patient with tumor recurrence had two foci of microscopic disease in the cervix and vagina. In addition, four of seven patients with lesions 6 cm or greater in size had squamous cell carcinoma antigen values in excess of 5.0 ng/ml.

Pretreatment serum concentrations of CA 125 and carcinoembryonic antigen were determined for the 30 patients with cervical carcinoma. CA 125 values ranged from 5.2 to 446.5 U/ml and were elevated in six (20%) patients. Carcinoembryonic antigen levels ranged from 0.3 to 78.5 ng/ml and were elevated in eight (27%) subjects. Pretreatment squamous cell carcinoma antigen and carcinoembryonic antigen values were both elevated in four (13%) of the 30 patients (Table II). All three antigens were elevated in three (10%) patients and were within the normal range in nine (30%) patients. The proportion of patients with elevated pretreatment levels (57%) of squamous cell carcinoma antigen was significantly higher than the proportion having elevated carcinoembryonic antigen levels (27%) or CA 125 levels (20%) ($p < 0.05$). The number of patients having elevated carcinoembryonic antigen levels compared with elevated CA 125 levels was not significantly different.

Serial serum samples were obtained from 16 patients

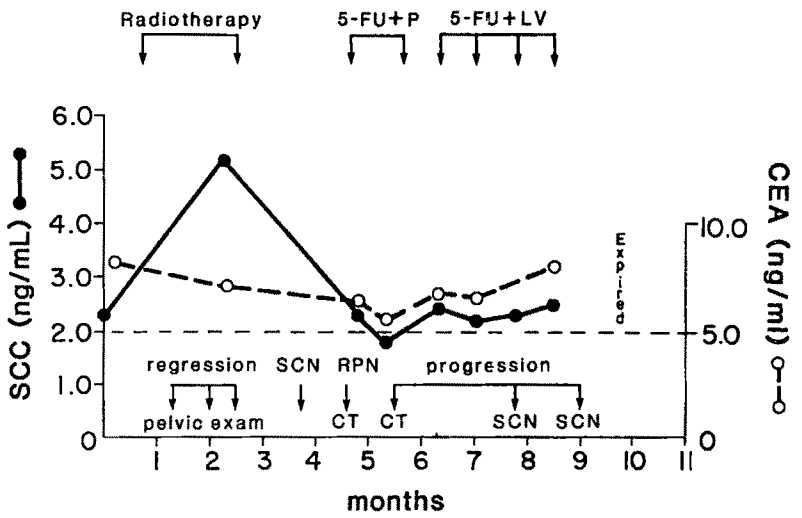


Fig. 2. Serum squamous cell carcinoma antigen values *(solid circles)* and carcinoembryonic antigen values *(open circles)* during therapy in a 51-year-old patient with a Stage IIIB cervical squamous cell carcinoma. The *dotted line* represents the upper limit for normal values of squamous cell carcinoma antigen (2.0 ng/ml) and carcinoembryonic antigen (5.0 ng/ml, smoker). *P* = Platinol; *LV* = leucovorin; *SCN* = supraclavicular adenopathy; *RPN* = retroperitoneal adenopathy, *CT* = computed tomography. See Fig. 1.

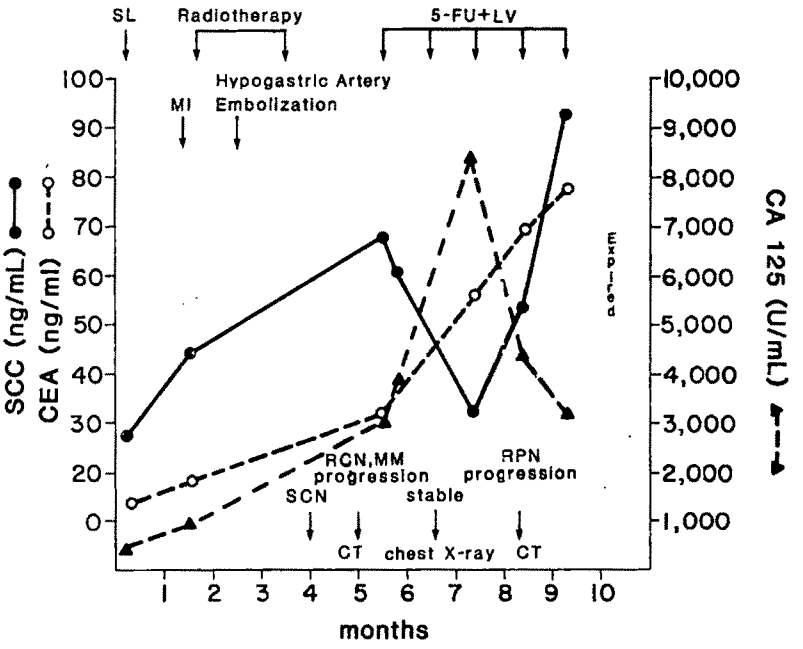


Fig. 3. Serum squamous cell carcinoma antigen concentrations *(solid circles)*, carcinoembryonic antigen levels *(open circles)*, and CA 125 levels *(solid triangles)* in a 42-year-old patient with a Stage IIIB cervical squamous cell carcinoma during irradiation and chemotherapy. *SL* = Staging laparotomy; *MI* = myocardial infarction; *RCN* = retrocrural adenopathy; *MM* = mediastinal mass; *CEA* = carcinoembryonic antigen. See Figs. 2 and 3.

with cervical carcinoma. Three to 11 specimens were available for each patient over a 2- to 15-month period. Pretreatment squamous cell carcinoma antigen levels were <2.0 ng/ml in seven of the 16 patients; antigen values remained within the normal range in four, rose transiently during radiotherapy in two, and increased to 4.9 ng/ml in one case coincident with lung and supraclavicular node metastases.

Pretreatment squamous cell carcinoma antigen values were elevated in nine of the 16 patients from whom

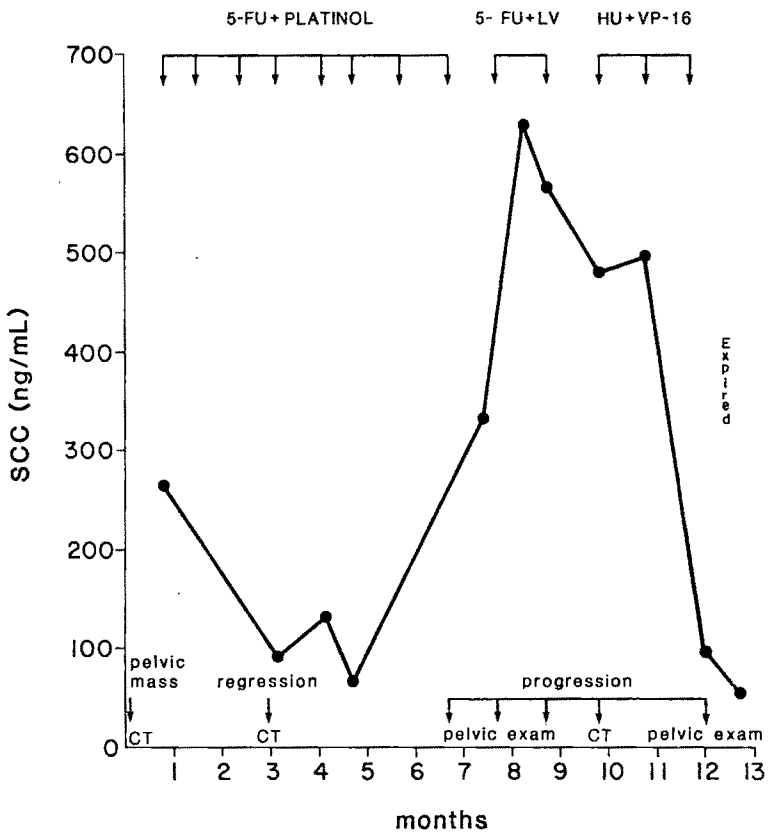


Fig. 4. Serial squamous cell carcinoma antigen levels during chemotherapy in a 32-year-old patient with recurrent cervical squamous carcinoma. *HU* = Hydroxyurea. See Figs. 1 through 3.

serial serum samples were collected. Eight patients had cervical squamous cell carcinoma and one had adenosquamous carcinoma. Two patients, one with a Stage IB and the other with a IIA lesion, have completed whole-pelvis external irradiation. Decline in squamous cell carcinoma antigen levels to <2.0 ng/ml occurred in both cases, at 11 and 27 Gy (1 Gy is equal to 100 rad), in association with regression of the visible and palpable tumor.

Two patients with Stage IB and IIIA lesions remain clinically free of disease 10 and 15 months after the initial diagnosis. Squamous cell carcinoma antigen levels fell to <2.0 ng/ml after therapy and remained within the normal limits. In contrast, Fig. 1 shows re-elevation of squamous cell carcinoma antigen values in one patient 12 months after the diagnosis of a Stage IIA cervical squamous cell carcinoma; however, recurrence could not be confirmed until 4 months after the elevated squamous cell carcinoma antigen level.

Two subjects received radiation therapy for bulky (diameter >6 cm) Stage IIB and IIIB cervical tumors (Figs. 2 and 3). Metastatic disease involving the supraclavicular nodes was confirmed 4 months after the initial diagnosis in both patients. With the administration of chemotherapy, squamous cell carcinoma antigen values decreased transiently in both patients and fell to within the normal limits in one. Squamous cell carcinoma antigen values stabilized to levels approaching 2.5 ng/ml in one patient and rose to a maximum of 92.2 ng/ml in the other. Both patients died of disease 10 months after the initial diagnosis.

In two patients with recurrent cervical carcinoma, pelvic masses were detected at 19 and 34 months after radiotherapy for Stage IIB and IB lesions (Figs. 4 and 5). In one case, serum squamous cell carcinoma antigen values fell from 265.4 ng/ml to 90.6 ng/ml (a level 45-fold greater than the reference value) after three courses of chemotherapy, coincident with tumor regression (Fig. 4). Squamous cell carcinoma antigen values increased and then fell to 51.4 mg/ml (a level 26-fold greater than the reference value) in association with progression of disease. In the second case, squamous cell carcinoma antigen levels fell to <2.0 ng/ml after four courses of chemotherapy (Fig. 5) in association with stabilization of disease. Elevated squamous cell carcinoma antigen levels were observed 5 months before evidence of progression.

Declining (Figs. 1 and 4) and rising (Figs. 2

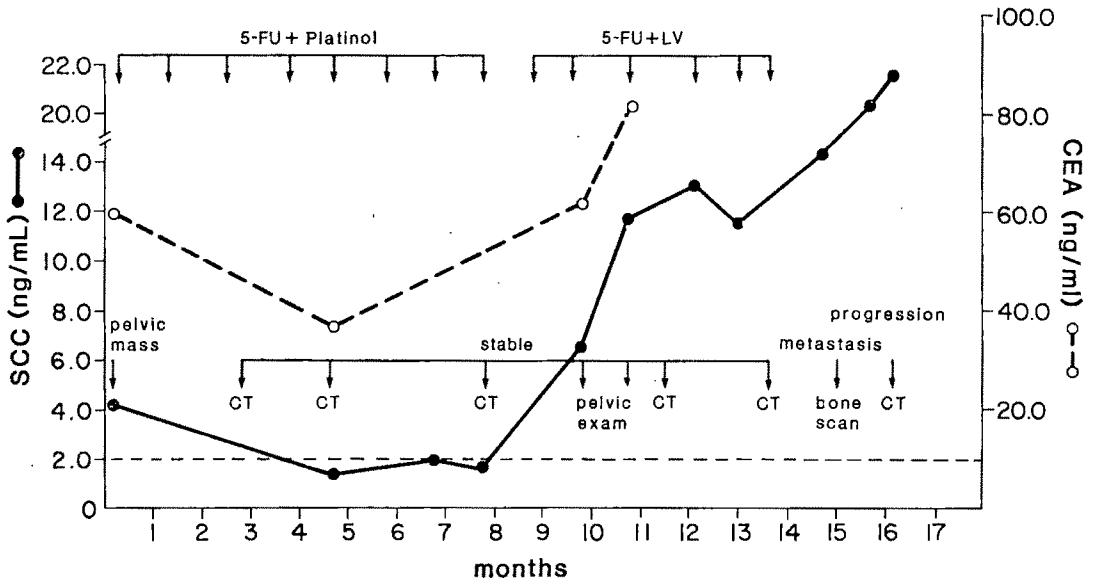


Fig. 5. Serum squamous cell carcinoma antigen concentrations *(solid circles)* and carcinoembryonic antigen *(open circles)* during chemotherapy in a 42-year-old patient with recurrent cervical adenosquamous carcinoma. The chemotherapy regimen was modified because of neurotoxicity. See Figs. 1 through 4.

through 5) squamous cell carcinoma antigen levels, respectively, corresponded with disease regression and progression. Elevated squamous cell carcinoma antigen levels anteceded clinical evidence of disease progression by 5 months in one case (Fig. 5) and antedated detectable recurrence by 4 months in another (Fig. 1). In one patient, three CA 125 values varied in parallel with paired squamous cell carcinoma antigen levels whereas the two antigens fluctuated in opposite directions for four values (Fig. 3). All but two of 17 paired carcinoembryonic antigen and squamous cell carcinoma antigen levels (Figs. 2, 3, and 5) varied in the same direction.

Comment

Clinical trials by Japanese investigators have demonstrated good agreement between serum TA-4 values and the extent,[1,6] prognosis,[7] and progression[3] of cervical squamous cell carcinoma. Normalization of TA-4 levels was observed to occur within 7 to 14 days after radical hysterectomy.[1,3] Furthermore, elevated TA-4 values preceded clinical evidence of recurrent disease by a mean of 3.6 months in 11 of 15 patients.[8]

Recently, Maruo et al.[4] reported the usefulness of measuring the subfraction of TA-4 in patients with cervical squamous cell carcinoma. In the Japanese population, 52% of 73 patients had elevated squamous cell carcinoma antigen levels (>2.5 ng/ml in the study by Maruo et al.[4]). A greater proportion of patients with advanced disease had elevated squamous cell carcinoma antigen levels relative to patients with early disease. Only 16% of Stage I versus 83% of Stage III tumors were associated with squamous cell carcinoma antigen values >2.5 ng/ml. Higher mean values of the antigen were reported in more advanced stage lesions. In addition, squamous cell carcinoma antigen levels fell below the reference value within 72 hours of radical hysterectomy in 15 of 16 patients, whereas in seven of eight subjects receiving radiotherapy, a transient rise in levels during the initial 20 Gy was followed by a decline to normal limits after 40 to 60 Gy.[4]

In this study, all 52 female donors, all 15 pregnant patients, and the 23 subjects with benign gynecologic disease had serum squamous cell carcinoma antigen levels <2.0 ng/ml. Therefore, consistently low levels of squamous cell carcinoma antigen were found in sera from healthy women and in patients with nonmalignant gynecologic tumors. Elevated pretreatment levels of squamous cell carcinoma antigen (>2.0 ng/ml in this study) were observed in 14% of 51 patients with cervical intraepithelial neoplasia and 67% of 24 patients with cervical squamous cell carcinoma. Squamous cell carcinoma antigen levels exceeded 2.5 ng/ml in 12 (50%) subjects with cervical squamous cell carcinoma. These findings are consistent with earlier studies by Japanese investigators. However, the test is not suitable for screening persons for invasive cervical carcinoma, since elevated levels occur in cases of cervical intraepithelial neoplasia and values <2.0 ng/ml are seen in subjects with cervical carcinoma.

Elevated squamous cell carcinoma antigen values were detected in four of eight patients with vaginal or vulvar squamous cell carcinoma. Additional investigation may demonstrate that the assay is useful in monitoring the clinical course of patients with these malignancies.

Among the 30 patients with cervical carcinoma, pretreatment squamous cell carcinoma antigen levels were elevated more frequently than were carcinoembryonic antigen levels (57% versus 27%); however, elevated carcinoembryonic antigen values were associated with normal squamous cell carcinoma antigen levels in 13% of these cases of cervical carcinoma. Therefore, carcinoembryonic antigen in conjunction with squamous cell carcinoma antigen determinations may be of value in monitoring patients with cervical carcinoma. Only 20% of patients had elevated CA 125 values, and in all patients elevated levels were accompanied by elevated carcinoembryonic antigen and/or squamous cell carcinoma antigen levels. No benefit was demonstrated for the assay of CA 125 in cases of cervical carcinoma.

In general, good agreement was found between squamous cell carcinoma antigen values and regression, progression, and recurrence of cervical carcinoma. Rising squamous cell carcinoma antigen values were associated with tumor progression (Figs. 2 through 5) or recurrence (Fig. 1) and preceded clinical evidence in two patients by 4 and 5 months (Figs. 1 and 5). Persistently elevated levels of serum squamous cell carcinoma antigen have been consistently associated with limited survival. Declining squamous cell carcinoma antigen levels were observed in six of nine cases of cervical cancer in association with objective tumor regression (Figs. 1, 2, and 4). However, declining values may also accompany disease progression or stabilization (Figs. 2 through 5). Furthermore, a fall in squamous cell carcinoma antigen values to <2.0 ng/ml may not always coincide with a tumor-free state (Figs. 2 and 5).

Elevated squamous cell carcinoma antigen values warrant thorough evaluation to delineate the presence and/or extent of disease. Rising squamous cell carcinoma antigen levels suggest the need for modification in the future course of therapy, namely, radical surgery and/or chemoradiotherapy for cure and/or discontinuation of ineffective chemotherapeutic regimens to avoid undue toxicity and permit the use of other agents for palliation.

Based on the few cases studied, rising levels of serum squamous cell carcinoma antigen appear to be predictive of progressive or recurrent disease while declining antigen values may coexist with regression, progression, or stabilization of tumor. Despite documentation of a downward trend in squamous cell carcinoma antigen levels, a highly elevated absolute value, several times greater than the reference value of 2.0 ng/ml, suggests the presence of a significant tumor burden. Squamous cell carcinoma levels within the normal range do not exclude the presence of tumor.

Although data from a larger number of cases of cervical carcinoma are necessary to establish guidelines for interpreting significant changes in serial levels of serum squamous cell carcinoma antigen, serum squamous cell carcinoma antigen determinations provide a noninvasive means of monitoring individual therapy in patients with cervical squamous cell carcinoma. Serum squamous cell carcinoma antigen concentrations obtained at intervals during initial therapy and thereafter may be of value in detecting persistent, progressive, or recurrent cervical carcinoma.

REFERENCES

1. Kato H, Morioka H, Aramaki S, et al. Radioimmunoassay for tumor-antigen of human cervical squamous cell carcinoma. Cell Mol Biol 1979;25:51-6.
2. Kato H, Torigoe T. Radioimmunoassay for tumor antigen of human cervical squamous cell carcinoma. Cancer 1977; 40:1621-8.
3. Kato H, Miyauchi F, Morioka H, et al. Tumor antigen of human cervical squamous cell carcinoma. Cancer 1979;43: 585-90.
4. Mauro T, Shibata K, Kimura A, et al. Tumor-associated antigen, TA-4, in the monitoring of the effects of therapy for squamous cell carcinoma of the uterine cervix. Cancer 1985;56:302-8.
5. Fleiss JL. Statistical methods for rates and proportions. New York: John Wiley and Sons, Inc, 1981:19-32.
6. Kato H, Morioka H, Tsutsui H, et al. Value of tumor-antigen (TA-4) of squamous cell carcinoma in predicting the extent of cervical cancer. Cancer 1982;50:1294-6.
7. Kato H, Morioka H, Aramaki S, et al. Prognostic significance of the tumor antigen TA-4 in squamous cell carcinoma of the uterine cervix. Am J Obstet Gynecol 1983;145:350-4.
8. Kato H, Kazunori T, Morioka H, et al. Tumor-antigen TA-4 in the detection of recurrence in cervical squamous cell carcinoma. Cancer 1984;54:1544-6.

Neonatal auditory acuity following in utero vibratory acoustic stimulation

G. Ohel, M.D., E. Horowitz, M.D., N. Linder, M.D., and H. Sohmer, M.D.
Jerusalem, Israel

The fetal heart rate response to externally applied vibratory acoustic stimulation is currently being evaluated as a test of fetal well-being. We have examined the safety of this procedure by subsequent testing of the auditory nerve and brain stem evoked responses in the neonates. The results demonstrated no differences in auditory acuity when control infants were compared with infants previously exposed to antenatal vibratory acoustic stimulation. (AM J OBSTET GYNECOL 1987;157:440-1.)

Key words: Vibratory acoustic stimulation, neonatal auditory functioning

The fetal heart rate (FHR) response to externally applied vibratory acoustic stimulation and its possible relevance to fetal well-being are currently being reevaluated.[1,2] The safety of antenatal vibratory acoustic stimulation in regard to subsequent neonatal auditory functioning has not been assessed carefully in the past. With this in mind, we have evaluated the auditory nerve and brain stem responses of neonates following their in utero exposure to vibratory acoustic stimulation.

The recording of auditory nerve and brain stem evoked responses to auditory stimuli is being used for the diagnosis of conditions such as hearing loss and neurological disorders at all ages, including neonates.[3] Details of the method were described elsewhere.[3] Briefly, the auditory stimuli were 10-second clicks, generated in earphones, from 80 dB in intensity above the threshold of the normal adult population down to 40 dB.

The responses were recorded as the potential difference between an earlobe and a scalp electrode. The electrical activity was filtered (bandpass 200 to 2000 Hz), amplified, and averaged by an average response computer (Microshev 2000). The auditory evoked potentials that are thus processed can determine the electrical response of the auditory system from the cochlea to the rostral brain stem. In the present study, we have evaluated several indices of normalcy from such recordings: a screening procedure for threshold, taken as the presence of replicable auditory nerve and brain stem evoked responses at 40 to 50 dB and latency measures of the auditory nerve and brain stem evoked responses to 80 dB clicks, including the latency of the first wave that is generated in the auditory nerve and the brain stem transmission time, which is a measure of the time interval between the auditory nerve response and a response from a more rostral region of the brain stem.[3]

From the Departments of Obstetrics and Gynecology and Pediatrics, Hadassah University Hospital, and the Department of Physiology, Hebrew University–Hadassah Medical School.
Received for publication June 30, 1986, revised September 24, 1986; accepted October 6, 1986.
Reprint requests: G. Ohel, M.D., Department of Obstetrics and Gynecology, Hadassah University Hospital, P. O. B. 12000, Jerusalem 91120, Israel.

Material and methods

Forty healthy neonates were examined 1 to 2 days after normal term deliveries. Twenty of these had been exposed to vibratory acoustic stimulation in labor. In the remaining cases, no such stimulation was received and these infants served as controls. For the stimulation, we have used a vibratory acoustic noise source, of a type used in some current clinical studies (Model 5B Electrolarynx, Western Electronic; audible sound 750 to 1000 Hz, output intensity 110 dB, vibrations between 110 and 200 Hz).[1,2] Three stimulations of 5 seconds' duration each were applied to the maternal abdomen overlying the fetal head, at the time of active labor. The FHR acceleration response was noted after each stimulation.

Results

The results of the neonatal auditory and brain stem evoked reponse studies are summarized in Table I. There were no differences in the first-wave latency times and the brain stem transmission times when the study and control neonates were compared (t test). In addition, both groups had similar auditory nerve and brain stem evoked response thresholds, which were never greater than 50 dB.

Comment

The presumed safety of fetal vibratory acoustic stimulation, which utilizes sound intensities of 110 dB, seems justified when the relatively high intrauterine background noise of about 70 dB and the attenuation of extramaternal sounds are considered. The present study, by use of an objective and sensitive test method, demonstrates no untoward effects on the auditory

Table I. Latency of auditory nerve response and brain stem transmission time in neonates after labor with and without vibratory acoustic stimulation

	Study group				*Control group*		
	Mean (msec)	*SD (msec)*	*No. of neonates*	*p Value*	*Mean (msec)*	*SD (msec)*	*No. of neonates*
Latency of first wave	1.53	0.18	20	NS*	1.56	0.18	20
Brain stem transmission time	5.71	0.28	20	NS*	5.71	0.27	20

*Student's *t* test.

nerve and brain stem responses of neonates after intrapartum vibratory acoustic stimulation. It is therefore felt that antepartum vibratory acoustic stimulation, as used in our study, is indeed a safe procedure.

REFERENCES

1. Ohel G, Simon A, Beyth Y, Sadovsky E. Intrapartum vibroacoustic stimulation in cases of normal and abnormal fetal heart rate patterns. Gynecol Obstet Invest 1986;21:1.
2. Smith CV, Phelan JP, Paul RH. Fetal acoustic stimulation testing. IV. The intrapartum assessment of fetal well being. In: Proceedings of the Sixth Annual Meeting of The Society of Perinatal Obstetricians, San Antonio, Texas, January 30–February 1, 1986.
3. Rubinstein A, Sohmer H. Latency of auditory nerve response in neonates one to eight hours old. Ann Otol Rhinol Laryngol 1982;91:205.

Breakage of a fetal scalp blade with retention of fragments in the infant's scalp

Phillip Nieburg, M.D., M.P.H., and Steven J. Gross, M.D.
Boston, Massachusetts, and Atlanta, Georgia

A term infant underwent scalp blood sampling just prior to delivery because of fetal tachycardia. Afterward, a portion of the scalp blade tip was missing and was noted in two fragments on x-ray film. Because of concern about the difficulty of removal, the fragments were left in situ. The infant progressed well. (Am J Obstet Gynecol 1987;157:441-2.)

Key words: Fetal scalp blade, retained fragments, management

As fetal scalp blood sampling has come into wider use for the assessment of fetal status during labor, complications of the procedure have been only infrequently reported. We would like to report a case of breakage of a fetal scalp blade with retention of the blade fragments in the infant's scalp.

Case report

A 3060 gm male infant was born to a 17-year-old, gravida 1, para 0 woman after an uncomplicated 38-week pregnancy. Labor lasted approximately 18 hours. Because of an apparent fetal tachycardia (160 to 190 bpm) noted after 12 hours of labor, membranes were ruptured and fetal blood for pH measurement was obtained by means of a scalp pH blade. At the termination of this procedure, a portion of the blade tip was noted to be missing.

Remainder of the 18-hour labor and delivery was uncomplicated. Fetal pH was 7.31. Apgar scores were 8 and 9 at 1 and 5 minutes, respectively, after a low-forceps vaginal delivery. Examination of the newborn infant was unremarkable. The scalp blood sampling site, although scabbed and easily visible, was not swollen or inflamed.

X-ray examination of the infant's skull, performed because of concern about the fractured blade, disclosed two radiopaque objects, each about 2 mm in longest dimension, lying over the right parietal bone, approximately 3 cm lateral to the sagittal suture (Fig. 1). One

From the Department of Pediatrics, Boston University School of Medicine, and the Division of Nutrition, Centers for Disease Control.
Received for publication October 31, 1986; March 10, 1987; accepted March 24, 1987.
Reprints not available.

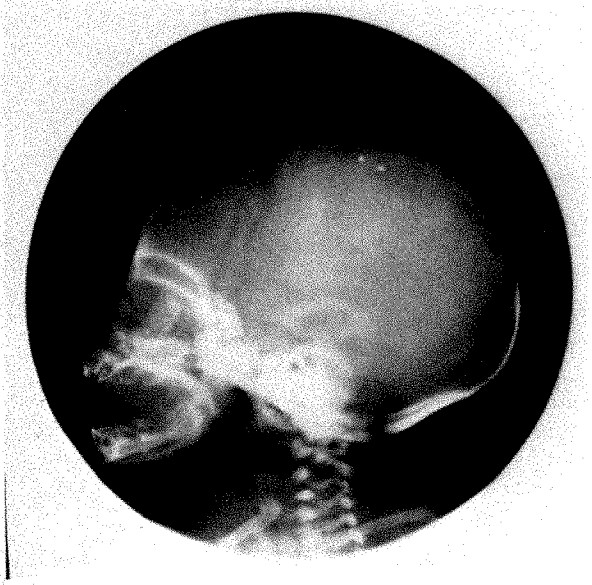


Fig. 1. X-ray film of infant's skull demonstrating two radiopaque blade fragments.

was 2.5 cm and the other 3 cm posterior to the coronal suture. A surgical consultant indicated that removal of these objects would be difficult and that, in view of their apparently inert nature, no indication for their removal existed. The parents were so advised.

The infant's subsequent hospital course was uneventful; he was discharged with his mother on the fourth postpartum day. He was progressing well on follow-up at 3 months of age.

Comment

Although recommendations have previously been made for careful scalp blade inspection after use,[1] we are unaware of previous published reports of retained blade fragments.

However, retention of spiral scalp electrode fragments in the scalp was reported by Thoulon and Gonnet.[2] A missing spiral electrode fragment was localized by x-ray film and removed with the infant under local anesthesia. Follow-up was uncomplicated. Lauritsen[3] reported a recurrent scalp abscess in a child admitted at 3½ months of age. An 8 mm scalp electrode fragment, not previously noted to be missing, was removed at the time of abscess incision and drainage.

An approach to the problem of retained blade fragment(s) other than that described above has been the gentle probing of the incision site with a small mosquito clamp to which has been applied a small magnet (such as that used to mix blood in a capillary tube). The magnetized probe may attract the metal fragment, which can then be gently lifted out of the scalp (Paul R, personal communication).

Experience with our case confirms that careful inspection of both fetal scalp blades and spiral electrodes is important after their use. In addition, evaluation of young infants with scalp abscesses should include x-ray examination for blade or electrode fragments if fetal scalp blood sampling or scalp electrode monitoring has occurred.

REFERENCES

1. Pritchard JA, McDonald PC. Williams' obstetrics. 16th ed. New York: Appleton-Century-Crofts, 1980:36.
2. Thoulon J-M, Gonnet C. Les accidents de l'electrocardiographie foetale directe (à propos d'une de l'electrode de scalp). J Gynecol Obstet Biol Reprod 1978;7:1257-60.
3. Lauritsen JG. Skalpabscesser hos speadbarn sekundaert til retineret caputelektrodestykke. Ugeskr Laeger 1978;140: 2445-6.

Review of causes of perinatal mortality in a regional perinatal center, 1980 to 1984

Arne Ohlsson, M.D., Andrew T. Shennan, M.B., and Toby H. Rose, M.D.
Toronto, Ontario, Canada

During 1980 to 1984, 279 deaths occurred among 15,306 births in a regional perinatal unit. Survival to discharge corrected for lethal malformations was 81% or better in infants with a birth weight above 749 gm. Congenital malformations (23.2%), infections (21.3%), asphyxia (19.8%), and hyaline membrane disease (11%) caused most perinatal deaths. (AM J OBSTET GYNECOL 1987;157:443-5.)

Key words: Perinatal mortality review, congenital malformations, infections, neutropenia, neonate

The purpose of this study was to review all perinatal deaths in a regional perinatal unit to indicate areas for improvements and research.

Material and methods

The Perinatal Unit at Women's College Hospital, Toronto, Ontario, Canada, serves both as a community hospital and as a level III perinatal facility for the Central East Region of Ontario, which has approximately 55,000 deliveries per year. Details of the perinatal management in the unit have been published previously.[1]

All perinatal deaths occurring in 15,306 in-hospital births of >20 weeks' gestation during 1980 to 1984 were reviewed. The cause of death was coded according to the International Classification of Diseases (ICD), 9th edition. The ICD code "extreme immaturity" was used only for appropriately grown neonates with a birth weight <600 gm, who died of a condition directly related to immaturity. "Definite infection" was defined as a clinical picture compatible with severe infection and growth of bacteria or virus in blood, cerebrospinal fluid, or tissue material at autopsy or a microscopic diagnosis of pneumonia. "Probable infection" was defined as a clinical picture compatible with serious infection, bacterial colonization, and neutropenia (mature neutrophils <1300/mm³) or leukocytosis (leukocytes >30,000/mm³) and no other obvious causes of death. The occurrence of air leaks (pneumothorax, pneumomediastinum, pneumopericardium) and cardiac dysrythmias were noted in all neonatal deaths.

From the University of Toronto Regional Perinatal Unit and the Department of Pathology, Women's College Hospital.
Received for publication November 24, 1986; revised January 20, 1987; accepted February 16, 1987.
Reprint requests: Arne Ohlsson, M.D., Regional Perinatal Unit at Women's College Hospital, 76 Grenville St., Toronto, Ontario, Canada M5S 1B2.

Results

The birth weight distributions of live births, stillbirths, early neonatal deaths, late neonatal deaths, and

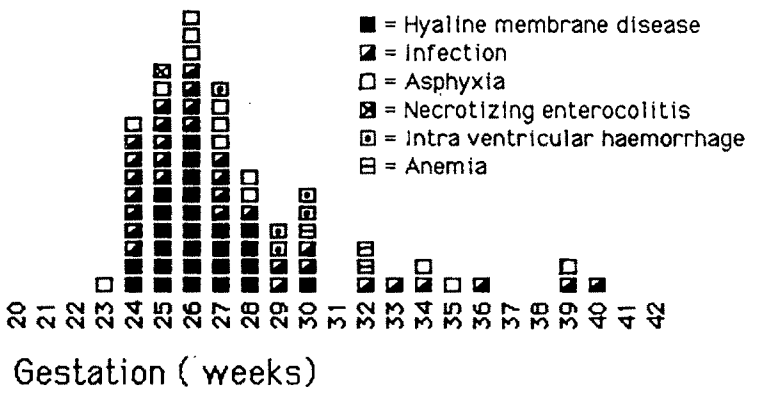


Fig. 1. Distribution of possibly avoidable early neonatal deaths by gestational age (each *square* represents one death). (Forty-three neonates with lethal congenital malformations, 24 with birth weight <600 gm, five with an "oligohydramnios sequence," and four with severe nonimmune hydrops were excluded.)

Table I. Perinatal mortality at Women's College Hospital, 1980 to 1984

	Birth weight					
	≤499 gm	*500-749 gm*	*750-999 gm*	*1000-1249 gm*	*1250-1499 gm*	*1500-1749 gm*
Live births	7	109	184	212	220	215
Stillbirths	3	19 (1*)	10 (1)	7	7 (1)	7 (1)
Total births	10	128	194	219	227	222
Early neonatal deaths	7 (1)	49 (2)	33 (3)	20 (7)	11 (5)	8 (6)
Late neonatal deaths	0	6	2	5 (1)	1	1
Infant deaths		3	4 (1)	1	3 (2)	
First-week survival (%)	0.0	55.1	82.1	90.6	95.0	96.3
Survival to discharge†	0.0	47.7	81.7	88.2	96.2	98.6

*Figures in parentheses represent deaths from congenital malformations.
†Corrected for lethal congenital malformations.

Table II. Cause of death (perinatal and infant deaths)

	Stillbirths		*Early neonatal deaths*		*Late neonatal deaths*		*Infant deaths*	
Cause	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Congenital malformations	11	12.9	43	27.6	7	31.8	7	43.8
Definite infections	17	20.0	22	19.9	8	36.4		
Probable infections			9					
Hyaline membrane disease			26	16.7	3	13.6		
Birth weight <600 gm (AGA)			24	15.4				
Asphyxia	38*	44.7	14	9.0				
Intraventricular hemorrhage			5	3.2	4	18.2		
Oligohydramnios sequence			5	3.2				
Hydrops fetalis	3	3.5	4	2.6				
Other	1	1.2	4	2.6				
Unknown	15	17.7						
Sudden infant death							1	6.3
Tracheal stenosis							1	6.3
Bronchopulmonary dysplasia							7	43.8
Total	85		156		22		16	

*Abruptio placentae, n = 15; intrauterine growth retardation, n = 8; cord complication, n = 8; infant of diabetic mother, n = 1; other, n = 6.

infant deaths and the survival rates are shown in Table I. The first-week survival rate increased from 15.6% below 600 gm to 71.4% in the 600 to 749 gm weight group. The distribution of early neonatal deaths that possibly could have been avoided according to gestational age is shown in Fig. 1. Multiple birth was associated with 13.3% of all deaths. The main causes of perinatal deaths and infant deaths are shown in Table II. The autopsy rate was 70%. Thirty-eight percent of the stillbirths occurred in hospitalized mothers. Air leaks occurred in 25.3% of the neonatal deaths. Renal failure causing hyperkalemia and cardiac tachydysrythmias occurred in 12.4% of early neonatal deaths <1000 gm. Early neonatal deaths from infections were associated with premature rupture of the membranes for >24 hours in 39% and infection in 67% of the mothers. Seventy-seven percent of the early neonatal deaths that were due to a definite infection involved neutropenia. Gram-negative bacteria were most commonly isolated (34%).

Comment

Prevention of preterm birth and low birth weight remains the major challenge in reproductive medicine today as mortality is closely linked to immaturity and low birth weight (Table I, Fig. 1). Multiple births carry a high risk of perinatal death. Events such as intraventricular hemorrhage, air leaks, and dysrythmias secondary to hyperkalemia often precede death in critically sick neonates.

The major cause of perinatal death was congenital malformation (23.2%). Many of these anomalies were detected antenatally, but very few conditions were potentially amenable to fetal operations. Elective abortion after early antenatal diagnosis is presently the only available approach to decrease this major cause of mor-

Birth weight				
1750-1999 gm	*2000-2249 gm*	*2250-2499 gm*	*2500+ gm*	*Total*
220	272	345	13,437	15,221
1 (1)	4 (1)	6 (1)	21 (4)	85
221	276	351	13,458	15,306
2 (2)	10 (9)	1	15 (8)	156
		2 (1)	5 (5)	22
4 (3)		1 (1)		16
99.1	96.3	99.7	99.9	
99.5	99.6	99.9	99.9	

tality. Routine use of ultrasonography at 17 and 33 weeks' gestation in all pregnant women[2] detects not only congenital anomalies but also other conditions associated with perinatal death such as multiple births and growth retardation.

It has been argued that with the advent of intensive care, perinatal mortality has been improved by moving inevitable deaths into the infant age group. In this study only 6% of the deaths occurred between 28 days of life and discharge to home, and the survival rates to discharge were minimally affected by late neonatal deaths or infant deaths (Table I). Given a birth weight of >749 gm and no congenital malformations the chance of survival to discharge to home was 81% or better.

The second most common cause of perinatal death was infection (21.3%). Neutropenia, known to be associated with poor outcome, occurred in 77% of the early neonatal deaths that were due to a definite infection. The incidence of maternal infection that was undetected prior to delivery was high in this group. Tests used so far to detect infectious morbidity in mothers with premature rupture of the membranes lack both sensitivity and specificity.

Hyaline membrane disease, infection, and asphyxia were the main, possibly preventable deaths (Fig. 1). Only one neonate born after 28 weeks' gestation died of hyaline membrane disease, but 10 died of infection. However, hyaline membrane disease remained the major cause of possibly preventable neonatal death at gestational ages below this (Fig. 1). Hyaline membrane disease also caused late neonatal deaths and was the initial diagnosis in seven infant deaths that were due to bronchopulmonary dysplasia and in one infant death due to tracheal stenosis. Bronchopulmonary dysplasia was the major cause of possibly preventable infant deaths.

This study represents a selected high-risk population of pregnant mothers admitted to a regional perinatal unit and should be complemented by an audit of all perinatal deaths in the geographic region served by the unit. Such an audit should also consider sociodemographic factors influencing pregnancy outcome. Randomized controlled studies are needed to determine the effectiveness of new interventions to prevent and/or treat preterm delivery, premature rupture of the membranes, low birth weight, maternal-fetal bacterial infections, hyaline membrane disease, and hyperkalemia.

REFERENCES

1. Milligan JE, Shennan AT. Perinatal management and outcome in the infant weighing 1000 to 2000 grams. Am J Obstet Gynecol 1980;136:269-75.
2. Persson P-H, Kullander S. Long-term experience of general ultrasound screening in pregnancy. Am J Obstet Gynecol 1983;146:942-7.

Mexiletine use in pregnancy and lactation

Holly E. Lownes, M.D., and Timothy J. Ives, Pharm.D.
Chapel Hill, North Carolina

Treatment of arrhythmias during pregnancy is complicated by insufficient information on the effects of the drug on the fetus or possible alterations of the drug's pharmacodynamics in the mother. The use of mexiletine, a newly introduced antiarrhythmic agent, during the entire course of pregnancy and subsequent lactation is presented. (Am J Obstet Gynecol 1987;157:446-7.)

Key words: Mexiletine, pregnancy, lactation, ventricular tachycardia

The fact that women continue to take medications during pregnancy and lactation is not a new observation; however, data on such drug use are limited for most agents. Paucity of such information, particularly for newly marketed drugs, may severely limit the therapeutic prerogatives of a physician when caring for a pregnant patient. Use during pregnancy of mexiletine (Mexitil, Boehringer Ingelheim, Ridgefield, Connecticut), a recently introduced type Ib antiarrhythmic agent, has been reported only twice in the medical literature.[1,2] In neither case, however, was mexiletine initiated prior to conception. We report on a patient who received mexiletine during the entire pregnancy and subsequent breast-feeding.

Case report

A 26-year-old woman, gravida 1, paro 0, presented at 13.5 weeks' gestation for prenatal care. Her past medical history was significant for mitral valve prolapse, first diagnosed at age 13, and ventricular tachycardia with multifocal ectopic beats. Control was poor over the years with various combinations of quinidine, phenytoin, propranolol, disopyramide, and flecainide. At age 24, she was begun on a regimen of mexiletine (200 mg three times per day) and atenolol (50 mg per day). The mexiletine dose was subsequently titrated to 250 mg three times per day; she remained on a regimen of that and atenolol, 50 mg per day, throughout her conception, pregnancy, and postnatal course.

Her prenatal course was complicated by two episodes of *Escherichia coli* urinary tract infections, both of which responded to ampicillin. There was a question of possible intrauterine growth retardation at 34 weeks' gestation, but this was not validated by two consecutive ultrasound studies (there was symmetric body-head growth consistent with 33 weeks at 35 weeks 4 days by dates). However, the fetus was thought to be small for gestational age. During her pregnancy, there was no apparent change in her dysrhythmia. A 24-hour Holter monitoring at 15 weeks' gestation showed two four-beat runs of ventricular tachycardia (145 to 225 bpm) with occasional to frequent multiform ventricular premature beats, including rare triplets, occasional to frequent pairs, and some interpolated, intermittent bigeminy and trigeminy. Furthermore, Holter monitoring revealed baseline ectopy and one four-beat run of ventricular tachycardia (110 bpm) at 24 weeks' gestation and one eight-beat burst of ventricular tachycardia (135 to 180 bpm) at 27 weeks. She continued to work until 35 weeks' gestation. Throughout the course of pregnancy, the patient experienced no adverse effects that were thought to be attributable to mexiletine.

At 39 weeks, she had premature rupture of the membranes. With epidural anesthesia and Pitocin induction, she had an uneventful 8.5-hour labor (6-hour first stage, 2.5-hour second stage) and vaginal delivery (no forceps required) of a healthy male infant with a birth weight of 2600 gm and Apgar scores of 9 and 10 at 1 and 5 minutes, respectively. Maternal cardiac monitoring during labor and delivery revealed the baseline and/or decreased ectopy. Serum mexiletine levels of the mother on admission and the child 9 hours post partum were <0.2 and 0.4 μg/ml, respectively (normal therapeutic range = 0.75 to 2.0 μg/ml).

The baby had a normal newborn course with heart rates of 120 to 160 bpm. At 17 days of life, he weighed 2155 gm and was feeding on breast milk only. It was felt that this was a case of failure to feed. With maternal education and formula supplementation, he regained his weight and had an acceptable growth curve. At 3 months of age, breast-feeding was discontinued. At 8 months, he was evaluated for seizure-like episodes. He had a normal neurological examination and a negative eletroencephalogram, but was found to have gastroesophageal reflux, which was diagnosed by an overnight pH probe and responded to corrective measures. He is currently 10 months old and in good health, with age-appropriate development.

From the Department of Family Medicine, School of Medicine, and the School of Pharmacy, University of North Carolina at Chapel Hill.
Received for publication October 8, 1986; accepted February 19, 1987.
Reprint requests: Timothy J. Ives, Pharm.D., Department of Family Medicine, 269H, University of North Carolina, Chapel Hill, NC 27514.

Comment

In most cases where atenolol has been used during pregnancy and the puerperium, the effects on the fetus, labor, and the neonate are minimal, although fetal bradycardia has been reported. Unlike atenolol, mexiletine is lipid soluble and freely crosses the placenta. Mexiletine is also excreted into breast milk with a mean breast milk/plasma ratio of 1.45,[2] indicating a higher drug concentration in milk than in the maternal plasma. Investigators[1] consider this concentration to be insufficient for measurement in the neonatal serum, but such was not the case in this patient. Although the American Academy of Pediatrics considers atenolol acceptable for use during breast-feeding, no such recommendation has as yet been made for mexiletine. Alternatives to breast-feeding should be considered while the mother is receiving mexiletine.

In our single experience, mexiletine did not appear to cause any long-term adverse consequences, but with the appearance of several disturbing situations (suboptimal growth during the first weeks post partum or failure to feed) clinicians should continue to exercise extreme caution with its use during pregnancy and lactation.

REFERENCES

1. Timmis AD, Jackson G, Holt DW. Mexiletine for control of ventricular dysrhythmias in pregnancy. Lancet 1980;2:647-8.
2. Lewis AM, Patel L, Johnston A, et al. Mexiletine in human blood and breast milk. Postgrad Med J 1981;57:546-7.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1987 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 156 (January-June) and Vol. 157 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Vasopressin-induced bradycardia in fetal and adult sheep is not dependent on an increase in blood pressure

Margaret A. Harper, M.D., and James C. Rose, Ph.D.
Winston-Salem, North Carolina

Other investigators have reported that intravenous infusion of synthetic arginine vasopressin into fetal lambs increases mean arterial pressure and decreases heart rate. To determine if the bradycardia produced by arginine vasopressin is a reflex response to the increase in blood pressure, we studied the effect of arginine vasopressin infusion on heart rate with and without blocking the increase in blood pressure. We performed 34 experiments in 12 chronically cannulated fetal lambs between 103 and 137 days' gestation. All animals had normal blood gas and pH values. Infusion of arginine vasopressin increased mean arterial pressure 10.1 ± 1.1 mm Hg and decreased heart rate 50 ± 8 bpm. Fetal heart rate decreased similarly when arginine vasopressin was infused and the hypertensive response was blocked with nitroprusside or a selective vasoconstrictor antagonist [1-(β-mercapto-β,β-cyclopentamethylene proprionic acid) 2-(0-methyl) tyrosine] arginine vasopressin. For comparison we also studied five adult nonpregnant ewes. Bradycardia was observed in the adults after infusion of arginine vasopressin alone and when the hypertensive response was blocked with the vasoconstrictor antagonist. We conclude that arginine vasopressin infusion causes a fall in heart rate independent of any increase in blood pressure in both the fetal lamb and the adult sheep. (Am J Obstet Gynecol 1987;157:448-53.)

Key words: Vasopressin, baroreceptor response, fetal lamb

Plasma concentrations of arginine vasopressin in sheep fetuses have been shown to be elevated in association with hypoxia, hemorrhage, and hypotension.[1-5] Intravenous infusion of arginine vasopressin into the sheep fetus causes an increase in blood pressure, a decrease in heart rate, and a redistribution of blood flow.[6-8] For these reasons, a role for arginine vasopressin in the fetal cardiovascular response to intrauterine stress has been suggested. The increase in blood pressure is due at least in part to direct peripheral vasoconstriction.[9] The mechanism of bradycardia has not been completely elucidated. Arginine vasopressin–induced bradycardia has been attributed to a direct effect of the peptide on the heart, a reflex response to the increase in blood pressure mediated by activation of the vagal efferents, inhibition of the cardioaccelerator mechanism, or a direct central effect on the cardioregulatory neurons.[10] The effect of an intravenous infusion of arginine vasopressin on heart rate while the pressor response is controlled has not previously been reported in the fetus. The present study was designed to determine what role, if any, increased blood pressure has in arginine vasopressin–induced bradycardia in the fetal lamb and adult sheep.

From the Department of Obstetrics and Gynecology and the Department of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest University.
This work was supported by National Institutes of Health Grant HD-17644.
Presented at the Thirty-third Annual Meeting of the Society for Gynecologic Investigation, Toronto, Ontario, Canada, March 19-22, 1986.
Received for publication November 3, 1986; revised January 21, 1987; accepted February 18, 1987.
Reprint requests: Margaret A. Harper, M.D., Department of Obstetrics and Gynecology, Bowman Gray School of Medicine, 300 South Hawthorne Road, Winston-Salem, NC 27103.

Material and methods

Animal preparation. We studied 12 chronically cannulated fetal lambs between 103 and 137 days' gestation and five adult nonpregnant ewes. Details of surgical procedures and animal maintenance have been described.[5] Briefly, ewes were deprived of food for 48 hours before operation. At operation the animals were anesthetized with ketamine and phenobarbital. In the pregnant ewes the uterus was exposed through a midline abdominal incision, the hind limbs of the fetus were delivered through a small uterine incision, and polyvinyl catheters were placed in a peripheral artery and vein and advanced into the aorta and inferior vena cava.

Table I. Pretreatment heart rate, mean arterial pressure, and blood gas values (mean ± SEM)

	n	*Heart rate*	*Mean arterial pressure*	*pH*	P_{CO_2}	P_{O_2}
Fetuses						
Arginine vasopressin	12	191 ± 8	42.1 ± 3.0	7.38 ± 0.02	41.6 ± 1.0	22.5 ± 1.7
Arginine vasopressin + vasoconstrictor antagonist	12	195 ± 9	44.0 ± 2.5	7.39 ± 0.02	42.4 ± 1.7	22.0 ± 0.8
Arginine vasopressin + nitroprusside	10	195 ± 9	39.7 ± 2.4	7.36 ± 0.01	44.4 ± 1.9	20.1 ± 1.0
Adults						
Arginine vasopressin	6	64 ± 4.2	70.2 ± 4.4	7.44 ± 0.05	33.9 ± 1.33	101.9 ± 8.64
Arginine vasopressin + vasoconstrictor antagonist	6	80 ± 5.3	75.5 ± 4.3	7.35 ± 0.06	33.0 ± 1.33	121.9 ± 12.64

An open-ended catheter was also inserted into the amniotic cavity for intrauterine pressure measurements. The aorta and vena cava of the pregnant ewe were cannulated via the left mammary artery and vein and the femoral artery and vein in the nonpregnant ewe. All maternal and fetal lines were filled with sterile heparin, closed with metal plugs, and exteriorized through a separate stab wound in the ewe's flank. The catheters were placed in a cloth pouch sutured to the ewe's skin. Five hundred milligrams of kanamycin was administered into the amniotic cavity on the day of operation, for the next 4 days, and then every other day. The ewe was given Combiotic (Pfizer), 0.1 ml · kg^{-1} intramuscularly on the day of operation, the next 4 days, and then every other day. Fresh heparin was placed in the vascular catheters daily. The animals were maintained in mobile metabolic carts.

Experimental protocol. All animals were allowed to recover at least 72 hours after operation before any experiments were undertaken. All experiments were done with the ewes awake and quiet. The vascular and amniotic catheters were opened and connected to Statham pressure transducers for monitoring fetal (in the pregnant animals) and adult (in the nonpregnant animals) phasic systolic and diastolic blood pressures and amniotic fluid pressures. Fetal and adult heart rates were obtained with cardiotachometers triggered by the arterial pulse wave. The pressures and heart rates were recorded continuously on a Beckman dynograph recorder. The animals were allowed a minimum of 30 minutes to acclimate to the experimental situation. All fetal arterial pressures reported below have been corrected for intra-amniotic pressure.

There were three experimental protocols as described below. Ten fetuses were studied in all three protocols. Two additional fetuses were studied in two protocols. All five nonpregnant ewes were studied in protocols 1 and 2. Initial placement in protocol 1, 2, or 3 was random. A total of 34 experiments were performed in the 12 fetuses, and 12 experiments were done in the five adult nonpregnant ewes. All experiments in the same animal were separated by at least 48 hours. Fetal weight was estimated according to the data of Joubert[11] for fetal weights at known gestational ages. Adult weight was obtained directly. Plasma vasopressin concentrations were measured by radioimmunoassay.[12]

Protocol 1: Arginine vasopressin infusion. Arginine vasopressin in sterile saline solution containing 0.1% bovine serum albumin was infused through a venous catheter at a rate of 8 ng $kg^{-1}min^{-1}$ for 30 minutes. The infused volume was constant in all fetuses at 0.103 ml min^{-1} and in all adults at 0.76 ml min^{-1}. Arterial blood (0.5 ml) was obtained just before the start of the infusion and at the end of the infusion for determination of blood gas, pH, and hematocrit. Blood gases were measured with a Radiometer blood analyzer with the use of appropriate electrodes. Heart rate and blood pressure were recorded continuously for 30 minutes prior to the beginning of the infusion, throughout the infusion, and for 1 hour after completion of the infusion.

Protocol 2: Arginine vasopressin infusion after pretreatment with an arginine vasopressin antagonist. The same procedure as in protocol 1 was carried out except that the animals were pretreated with a selective vasoconstrictor antagonist [1-(β-mercapto-β,β-cyclopentamethylene proprionic acid) 2-(0-methyl) tyrosine] arginine vasopressin, 30 to 175 μg kg^{-1}. The vasoconstrictor antagonist was given by intravenous bolus 15 minutes before the start of the arginine vasopressin infusion.

Protocol 3: Arginine vasopressin infusion with simultaneous infusion of sodium nitroprusside to control blood pressure. The same procedure as in protocol 1 was carried out except that sodium nitroprusside (250 μg/ml) was infused through a second venous line simultaneously with the arginine vasopressin infusion. The rate of infusion of sodium nitroprusside was adjusted in each

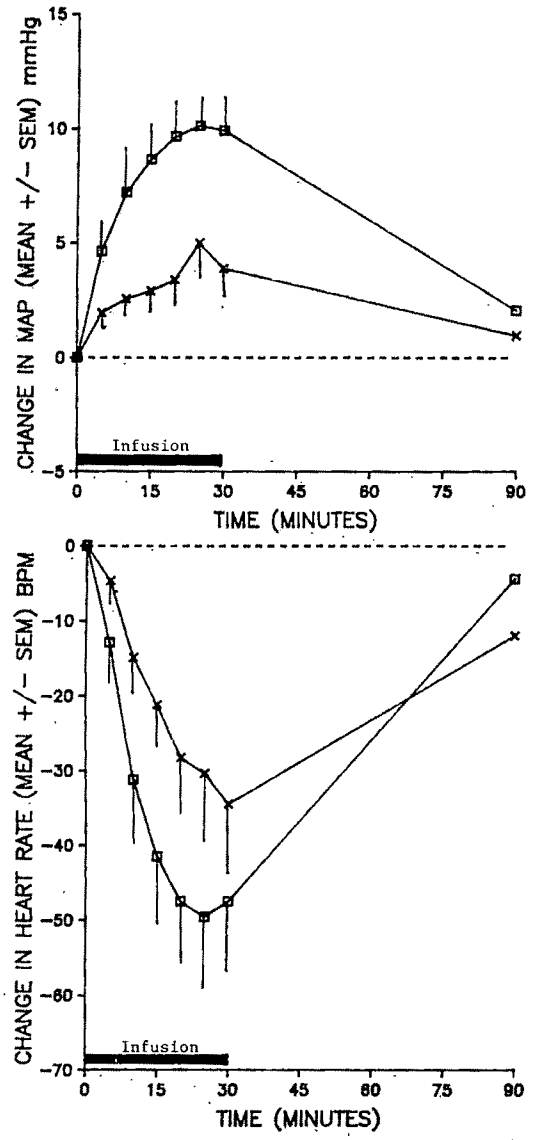


Fig. 1. Fetuses. *Top:* Change in mean arterial pressure *(MAP)* (mean ± SEM) with arginine vasopressin infusion alone (—□—) and with arginine vasopressin infusion after pretreatment with vasoconstrictor antagonist (—x—). Time error: F = 13.816, p = <0.001. Treatment error, F = 19.689, p = 0.001. *Bottom*: Change in heart rate (mean ± SEM) with arginine vasopressin infusion alone (—□—) and with arginine vasopressin infusion after pretreatment with vasoconstrictor antagonist (—x—). Time error: F = 14.450, p = <0.001. Treatment error: F = 1.733, p = .219.

animal to keep mean arterial pressure constant during the 30-minute arginine vasopressin infusion. Infusion rates ranged from 1.14 to 41.18 μg $kg^{-1} \cdot min^{-1}$.

Data analysis. The change in mean arterial pressures and heart rates were analyzed by use of two-way analysis of variance with repeated measures design. Hematocrit, blood gas values, resting heart rates, and mean arterial pressures were compared by means of the *t* test. All values are recorded as mean ± SEM.

Results

In both the fetuses and adults, resting heart rate, mean arterial pressure, arterial blood gas values, and hematocrits were not different among the different treatment groups (Table I) except resting heart rate in the adult arginine vasopressin treatment group was lower than resting heart rate in the arginine vasopressin plus arginine vasopressin antagonist treatment group (p = 0.035). Arterial blood gases and hematocrits did not change significantly during the infusions (data not shown). Plasma arginine vasopressin levels in the fetuses increased from a resting control value of 5.2 ± 1.1 to 113.2 ± 40.7 pg ml^{-1} at the end of the 30-minute infusion.

When arginine vasopressin alone was infused in the fetuses, mean arterial pressure increased 10.1 ± 1.1 mm Hg (Fig. 1). Administration of the vasoconstrictor antagonist did not alter fetal resting mean arterial pressure but did attenuate the pressor response to arginine vasopressin. Two-way analysis of variance revealed a significant effect of time (p < 0.001) and a significant difference between the two treatment groups (p = 0.001).

With arginine vasopressin alone, heart rate fell 50 ± 8 bpm. The vasoconstrictor antagonist did not change resting fetal heart rate and after pretreatment with the antagonist arginine vasopressin still produced a profound bradycardia that was not different from that observed when arginine vasopressin was infused alone.

When simultaneous infusions of sodium nitroprusside and arginine vasopressin were given to 10 fetuses, mean arterial pressure actually fell at 5, 10, 15, and 30 minutes (Fig. 2). By two-way analysis of variance, the change in mean arterial pressure was significantly different when compared with that associated with arginine vasopressin alone (p = 0.001). Heart rate still fell in this group and was not statistically different from the fall in heart rate with arginine vasopressin alone (p = 0.196).

Six pairs of experiments were carried out in five adult nonpregnant ewes for comparison (Fig. 3). Mean arterial pressure increased 18.6 ± 3.0 mm Hg with arginine vasopressin alone. After pretreatment with the vasoconstrictor antagonist, the peak increase in mean arterial pressure was 4.1 ± 1.6 mm Hg. Heart rate fell 30 ± 7 bpm with arginine vasopressin alone and 18 ± 5 bpm after pretreatment with the vasoconstrictor antagonist. By two-way analysis of variance, there was a significant effect of time on mean arterial pressure and a significant difference in the change in mean arterial pressure between the two treatment groups. There was also a significant effect of time on heart rate. There was no difference in fall in heart rate between the two treatment groups.

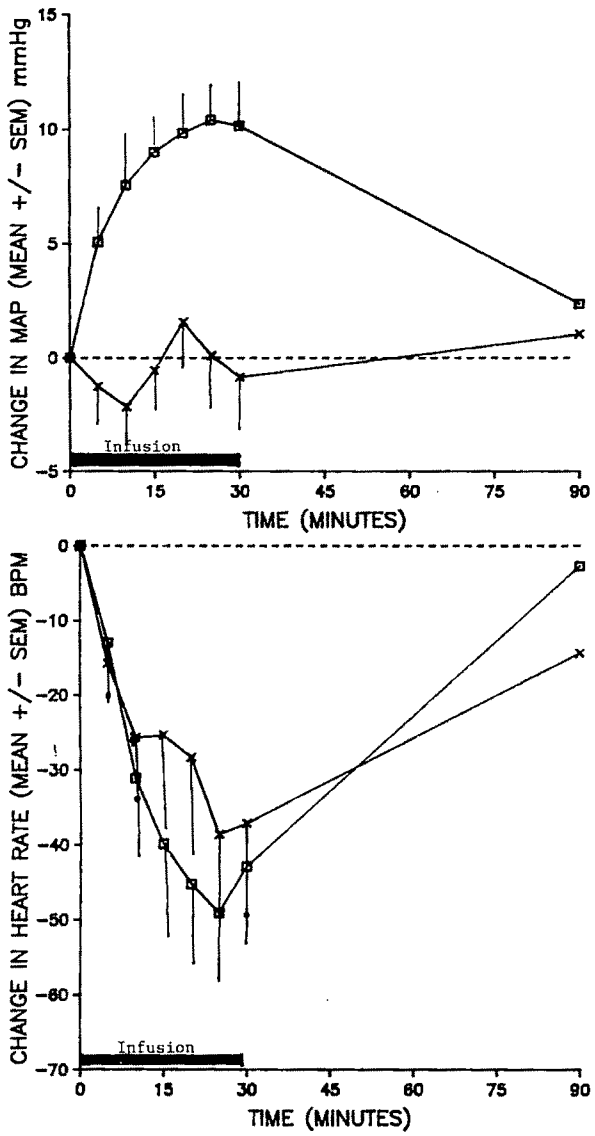


Fig. 2. Fetuses. *Top:* Change in mean arterial pressure *(MAP)* (mean ± SEM) with arginine vasopressin infusion alone (—□—) and with arginine vasopressin with nitroprusside (—x—). Time error: F = 2.231, p = 0.05. Treatment error: F = 233.54, p = 0.001. *Bottom:* Change in heart rate (mean ± SEM) with arginine vasopressin infusion alone (—□—) and with arginine vasopressin with nitroprusside (—x—). Time error: F = 10.020, p = 0.001. Treatment error: F = 0.553, p = NS.

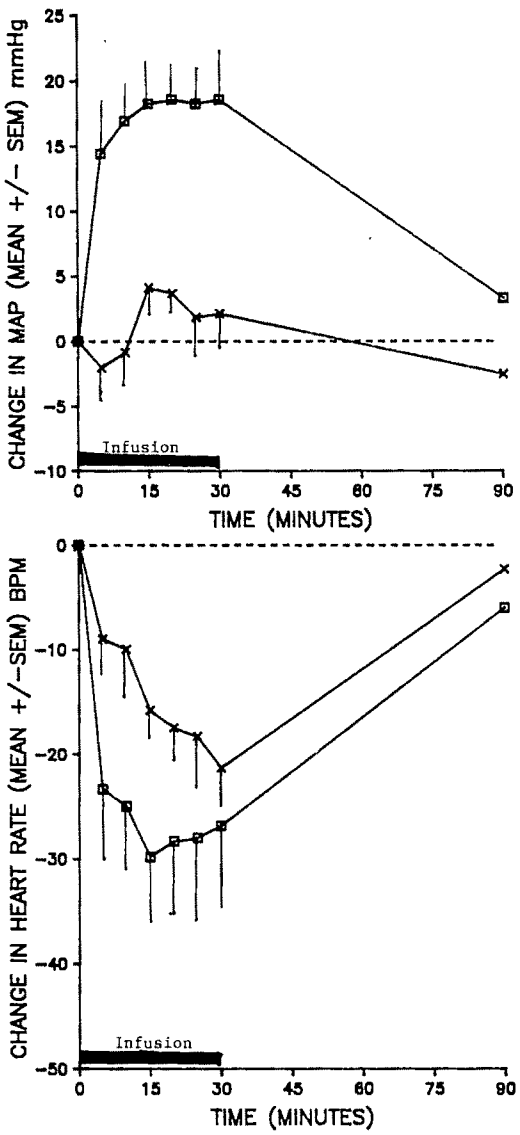


Fig. 3. Adults. *Top:* Change in mean arterial pressure *(MAP)* (mean ± SEM) with arginine vasopressin infusion alone (—□—) and with arginine vasopressin infusion after pretreatment with vasoconstrictor antagonist (—x—). Time error: F = 13.394, p = 0.05. Treatment error: F = 18.051, p = 0.008. *Bottom:* Change in heart rate (mean ± SEM) with arginine vasopressin infusion alone (—□—) and with arginine vasopressin infusion after pretreatment with vasoconstrictor antagonist (—x—). Time error: F = 9.591, p = <0.001. Treatment error: F = 3.445, p = 0.121.

Comment

Vasopressin increases arterial pressure and decreases heart rate when administered intravenously to fetuses.[6-8] Various stressful stimuli cause an increase in plasma concentrations of arginine vasopressin in the fetus, and it has been suggested that arginine vasopressin is a mediator of bradycardia associated with fetal distress.[7, 13] Our results demonstrate that, in both fetuses and adults, arginine vasopressin can produce bradycardia in the absence of an increase in blood pressure. This indicates that the bradycardia produced by arginine vasopressin is not simply a reflex response to an increase in arterial pressure. The observation is compatible with the report by Heyndrickx et al.[14] that arginine vasopressin, unlike angiotensin, reduced heart rate after denervation of arterial baroreceptors in conscious dogs. In addition, Varma et al.[10] also observed a pronounced bradycardia with arginine vasopressin in-

fusion in anesthetized dogs when systemic arterial pressure was controlled with a mechanical device.

The mechanism whereby arginine vasopressin can decrease heart rate when arterial pressure is held constant is not clear; however, it is known that arginine vasopressin can decrease heart rate in dogs in which both vagi are cut.[15] This suggests that peripheral infusions of arginine vasopressin can decrease cardioaccelerator activity. Such an effect may be mediated centrally since arginine vasopressin administered into the fourth ventricle produces no change in blood pressure but a decrease in heart rate in anesthetized dogs.[10]

Other possible mechanisms for the bradycardia associated with increased levels of arginine vasopressin include a direct negative chronotropic effect on the myocardial cells or myocardial ischemia due to coronary constriction. Scott et al.[16] found no significant differences between rat myocardial contraction frequency in tissue culture measured before and after addition of vasopressin. Myocardial blood flow in the fetal lamb has been reported to be unaffected during arginine vasopressin–induced bradycardia.[6]

Resting arginine vasopressin levels in the fetuses we studied (5.2 ± 1.1 pg $\cdot$ ml^{-1}) were similar to those of previous reports (4.81 ± 1.2 and 8.1 ± 2.6 pg $\cdot$ ml^{-1}).[6, 8] The mean arginine vasopressin clearance calculated from eight fetuses was 74.8 ml $\cdot$ kg^{-1} $\cdot$ min^{-1} and is also comparable to that of previous reports (60.5 ml $\cdot$ kg^{-1} $\cdot$ min^{-1}).[7]

The increase in mean arterial pressure of 10.1 ± 1.1 mm Hg and the decrease in heart rate of 50 ± 8 bpm with arginine vasopressin infusion in the fetuses are in agreement with the observations of others.[6-8]

The percent changes in mean arterial pressure in the fetuses and adult ewes were similar (23.8 versus 26.5). However, the percent change in heart rate was 47.6 in the adults versus 26.2 in the fetuses. This suggests that, while there is no difference in relative responsiveness to the pressor effects of arginine vasopressin between fetus and adult, the fetus is less sensitive to the negative chronotropic effect of arginine vasopressin. The adult animal is better able to maintain cardiac output by increasing stroke volume when heart rate falls than is the fetus.[17] This relative decrease in sensitivity may therefore be protective for the fetus.

Mirro et al.[18] observed that nitroprusside infusion in newborn lambs caused no change in heart rate while producing a significant decrease in mean arterial pressure. Nitroprusside causes an increase in heart rate in humans when used to control blood pressure[19] and has been shown to have no direct cardiac effects in dogs.[20] We therefore conclude that the bradycardia observed in these ovine fetuses given nitroprusside and arginine vasopressin simultaneously was an effect of arginine vasopressin.

In both the fetal and adult sheep the bradycardia produced by arginine vasopressin was not blocked by the vasoconstrictor antagonist. In contrast, Schwartz et al.[21] found in unanesthetized dogs that arginine vasopressin in the presence of the vasoconstrictor antagonist caused an increase in heart rate. They used a higher dose of arginine vasopressin (10 ng $\cdot$ kg^{-1} $\cdot$ min^{-1} for 60 minutes versus 8 ng $\cdot$ kg^{-1} $\cdot$ min^{-1} for 30 minutes) and a smaller dose of the vasoconstrictor antagonist (10 versus 30 to 175 μg $\cdot$ kg^{-1}). The pressor response was blocked in their animals with the vasoconstrictor antagonist. The differences in observations are probably due to species differences. It would appear that while in dogs the antidiuretic activity of arginine vasopressin produces cardiovascular effects opposite those produced by its vasoconstrictor action, this is not the case in sheep.

There was no significant effect of the vasoconstrictor antagonist on blood gas values, heart rate, or mean arterial pressure, indicating that vasopressin probably does not play a major role in circulatory regulation in the resting state.

REFERENCES

1. Rurak DW. Plasma vasopressin levels during hypoxaemia and the cardiovascular effects of exogneous vasopressin in foetal and adult sheep. J Physiol (Lond) 1978;277:341.
2. Daniel SS, Stark RI, Zubrow AB, Fox HE, Husain MK, James LS. Factors in the release of vasopressin by the hypoxic fetus. Endocrinology 1983;113:1623.
3. Alexander DP, Britton HG, Forsling ML, Nixon DA, Ratcliffe JG. Pituitary and plasma concentrations of adrenocorticotrophin, growth hormone, vasopressin, and oxytocin in fetal and maternal sheep during the later half of gestation and the response to hemorrhage. Biol Neonate 1974;24:206.
4. Drummond WH, Rudolph AM, Keil LC, Gluckman PD, MacDonald AA, Heymann MA. Arginine vasopressin and prolactin after hemorrhage in the fetal lamb. Am J Physiol 1980;238(Endocrinol Metab 1):E214.
5. Rose JC, Meis PJ, Morris M. Ontogeny of endocrine (ACTH, vasopressin, cortisol) response to hypotension in lamb fetuses. Am J Physiol 1981;140(Endocrinol Metab 3):E656.
6. Iwamoto HS, Rudolph AM, Keil LC, Heymann MA. Hemodynamic responses of the sheep fetus to vasopressin infusion. Circ Res 1979;44:430.
7. Wiriyathian S, Porter JC, Nadan RP, Rosenfeld CR. Cardiovascular effects and clearance of arginine vasopressin in the fetal lamb. Am J Physiol 1983;245(Endocrinol Metab 8):E24.
8. Tomita H, Brace RA, Chung CY, Longo LD. Vasopressin dose response effects on fetal vascular pressures, heart rate and blood volume. Am J Physiol 1985;249(Heart Circ Physiol 18):H974.
9. Schmid PG, Abboud FM, Wendling MG, et al. Regional vascular effects of vasopressin: plasma levels and circulatory responses. Am J Physiol 1974;227:998.
10. Varma S, Jaju BP, Bhargava KP. Mechanism of vasopressin-induced bradycardia in dogs. Circ Res 1969;24:787.
11. Joubert DM. A study of prenatal growth and development in the sheep. J Agric Sci 1956;47:382.
12. Rose J, Morris M, Meis P. Hemorrhage in newborn lambs: effects on arterial blood pressure, ACTH, cortisol and vasopressin. Am J Physiol 1981;54:E585.

13. Parboosingh J, Lederis K, Singh N. Vasopressin concentration in cord blood: correlation with method of delivery and cord pH. Obstet Gynecol 1982;60:179.
14. Heyndrickx GR, Boettcher DH, Vatner SF. Effects of angiotensin, vasopressin and methoxamine on cardiac function and blood flow distribution in conscious dogs. Am J Physiol 1976;231:1579.
15. Cowley A, Merrill D, Osborn J, Barber BJ. Influence of vasopressin and angiotensin on baroreflexes in the dog. Circ Res 1984;54:163.
16. Scott JA, Konstam M, Kolodny GM. Absence of a direct chronotropic effect of vasopressin on the myocardial cell. Pharmacology 1982;24:57.
17. Heymann MA. Fetal cardiovascular physiology. In: Creasy RK, Resnik R, eds. Maternal-fetal medicine: principles and practice. Philadelphia: WB Saunders, 1984:265-7.
18. Mirro R, Milley JR, Holzman IR. The effects of sodium nitroprusside on blood flow and oxygen delivery to the organs of the hypoxemic newborn lamb. Pediatr Res 1985;19:15.
19. Fremes SE, Weisel RD, Mickle DA, et al. A comparison of nitroglycerin and nitroprusside. I. Treatment of postoperative hypertension. Ann Thorac Surg 1985;39:53.
20. Chiba S, Kozu TM, Watanabe H. Analysis of cardiac action of nitroprusside in intact dogs and isolated atria. Jpn Heart J 1982;23:613.
21. Schwartz J, Liard JF, Ott C, Cowley AW. Hemodynamic effects of neurohypophyseal peptides with antidiuretic activity in dogs. Am J Physiol 1985;249(Heart Circ Physiol 18):H1001.

In vivo and in vitro modulation of growth hormone and prolactin of a mixed somatotropic-lactotropic pituitary microadenoma

Eberhard K. Muechler, M.D., Ko-En Huang, M.D. Joseph V. McDonald, M.D., and Loretta Tang, Ph.D.
Rochester, New York

Both in vivo and in vitro responses of prolactin and growth hormone to stimuli were studied in an acromegalic, amenorrheic woman with a chromophobe adenoma of the pituitary gland. Preoperative testing revealed a prolactin rise after thyrotropin-releasing hormone and chlorpromazine and a decline after L-dopa and bromocriptine administration. During 3.5-hour incubations, cultured tumor cells produced significant increases of growth hormone and prolactin after dibutyryl adenosine 3',5'-cyclic monophosphate stimulation, while bromocriptine inhibited both hormones. (Am J Obstet Gynecol 1987;157:453-5.)

Key words: Mixed pituitary microadenoma, acromegaly, amenorrhea, growth hormone, prolactin

Recent studies have shown that hyperprolactinemia is found in 42% to 70% of all acromegalic patients. We observed a young woman with amenorrhea, headaches, increase in hand and foot size, and coarsening of facial features caused by a mixed pituitary microadenoma that produced both prolactin and growth hormone.

Case report

A 32-year-old, gravida 2, para 2 woman was referred because of secondary amenorrhea of 7 years' duration. She complained of occasional episodes of headaches and denied galactorrhea. For the last 2 years prior to referral the weight of the woman increased by 20 pounds, height increased by 2 inches, and shoe size increased by two numbers. Facial features were coarse and puffy. Watery fluid could be expressed from the left breast. Baseline laboratory studies included: prolactin, 81 ng/ml (normal 0 to 35 ng/ml); thyroxine, 9.7 μg/dl (normal 5 to 13 μg/dl); thyroid-stimulating hormone 3.3 μU/ml (normal 1 to 10 μU/ml); growth hormone, 13 ng/ml (normal 0 to 11 ng/ml); follicle-stimulating hormone, 4.8 mIU/ml (normal 2 to 20 mIU/ml); luteinizing hormone, 11.6 mIU/ml (normal 4 to 30 mIU/ml). Polytomography of the sella turcica and computed tomographic scanning showed a markedly enlarged volume with cortical erosion and severe slanting of the left sella floor. Visual field examination was normal. Three months later the patient underwent transsphenoidal removal of a pituitary chromophobe adenoma with a focal acidophilic component. Four weeks after operation a weight loss of 11

From the Departments of Obstetrics and Gynecology and Neurosurgery, University of Rochester School of Medicine and Dentistry.
This work was supported in part by National Institutes of Health Grant HD-10837.
Received for publication January 15, 1987; accepted March 3, 1987.
Reprint requests: Eberhard K. Muechler, M.D., 601 Elmwood Ave., Rochester, NY 14642.

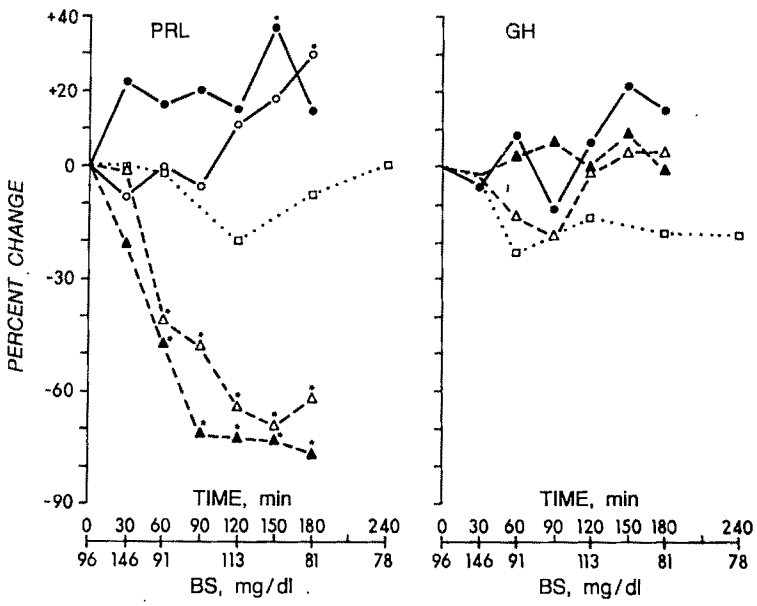


Fig. 1. Responses of prolactin *(PRL)* and growth hormone *(GH)* to thyrotropin-releasing hormone (●——●), bromocriptine (▲- - - -▲), L-dopa (△- - - -△), and glucose (□- - - -□). The response to chlorpromazine was tested for prolactin only (○——○). * = Statistically significant difference ($p < 0.05$).

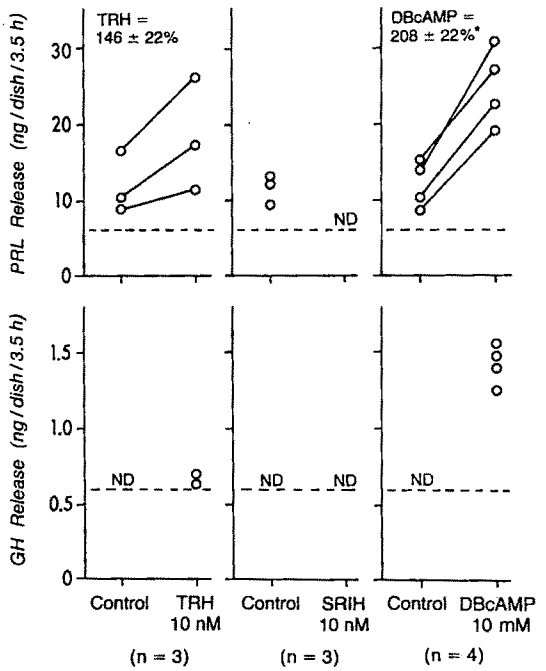


Fig. 2. Effect of thyrotropin-releasing hormone *(TRH)*, somatostatin *(SRIH)*, and dibutyrylcyclic adenosine monophosphate *(dbcAMP)* on prolactin *(PRL)* and growth hormone *(GH)* release by cultured cells. Day 4 cultures were incubated for two consecutive 3.5-hour periods. The initial incubation was in Dulbecco's modified Eagle's medium alone (control); the subsequent incubation was in Dulbecco's modified Eagle's medium containing 10 nmol/L thyrotropin-releasing hormone, 10 nmol/L somatostatin, or 10 nmol/L dibutyrylcyclic adenosine monophosphate. * = Significantly different from control period ($p < 0.05$). *Dotted line* indicates sensitivity of assay. *ND,* Not detectable.

pounds was observed, in addition to decreased puffiness of the face and all extremities and absence of headaches. Laboratory data were repeated at this time: growth hormone, 4.3 ng/ml; prolactin, 54 ng/ml; thyroxine, 9.5 μg/dl; thyroid-stimulating hormone, 3.1 μU/ml; follicle-stimulating hormone, 7 mIU/ml; luteinizing hormone, 6.3 mIU/ml. Seven months after operation amenorrhea was persistent. Headaches were lower in frequency and diminished in intensity when compared with those of the preoperative period. Prolactin had decreased further to a level of 30 ng/ml and growth hormone was not detectable.

Material and methods

On four consecutive days the patient was given test substances after an overnight fast. Serial blood samples were collected through an indwelling polyethylene catheter at 0, 30, 60, 120, 180, and 240 minutes after 100 gm of glucose was given orally. Blood samples were collected at −15, 0, 30, 60, 120, and 180 minutes after 500 mg of L-dopa orally, 0.5 mg of thyrotropin-releasing hormone intravenously, 2.5 mg of bromocriptine orally, and 25 mg of chlorpromazine intramuscularly. Growth hormone, prolactin, and thyroid-stimulating hormone were measured by established radioimmunoassays in the clinical laboratory of Strong Memorial Hospital in Rochester, New York. A portion of the removed pituitary gland was used to prepare monolayer cultures as described previously.[1] Concentrations of prolactin and growth hormone in the incubation media were determined by the double-antibody method of radioimmunoassay with materials

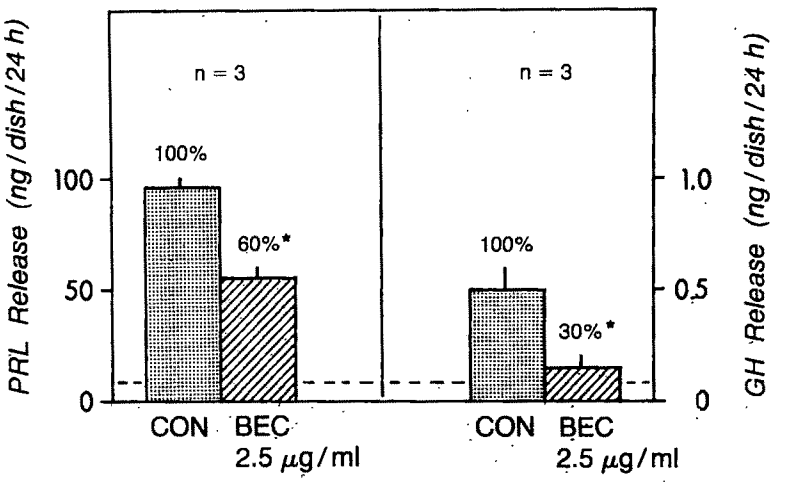


Fig. 3. Effect of bromocriptine *(BEC)* on prolactin *(PRL)* and growth hormone *(GH)* release by cultured cells. Day 14 cultures were incubated for two consecutive 24-hour periods. The initial control incubation was in Dulbecco's modified Eagle's medium supplemented with sera (10% horse serum, 5% fetal calf serum); the subsequent incubation was in the serum containing Dulbecco's modified Eagle's medium plus bromocriptine (2.5 μg/ml).

provided by the National Pituitary Agency, National Institutes of Health. Specific antibodies for prolactin and growth hormone were AFP-Ab-hRPL#1 and NIAMD-A-hGH (guinea pig), respectively.

Results

The in vivo response of thyroid-stimulating hormone to thyroid-releasing hormone was normal (baseline thyroid-stimulating hormone, 1.2 μU/ml; peak thyroid-stimulating hormone, 9.6 μU/ml after 30 minutes). All other in vivo results are summarized in Fig. 1. Cell cultures of tumor tissue were established and exposed to somatostatin, thyrotropin-releasing hormone, dibutyrylcyclic adenosine monophosphate, and bromocriptine. In vitro responses of prolactin and growth hormone to various agents are summarized in Figs. 2 and 3.

Comment

This study correlates in vivo and in vitro testing of pituitary function in a young woman with acromegaly and amenorrhea due to a mixed pituitary microadenoma. There was no change in growth hormone levels in vivo in response to L-dopa, bromocriptine, thyrotropin-releasing hormone, and glucose tolerance testing. Several other studies have shown that growth hormone is suppressed in acromegalic subjects by dopaminergic agonists such as L-dopa and bromocriptine. The known suppressive effect of dopaminergic drugs has led to long-term treatment with bromocriptine as an acceptable choice for treating acromegaly. The clinical and biochemical responses to surgical tumor ablation were satisfactory. The preoperative in vivo response of growth hormone and prolactin to stimuli was compared to in vitro responses of cultured cells. We observed a more consistent response of lactotropic cell function than of somatotropic cell function. While dibutyryl-cyclic adenosine monophosphate exhibited stimulating effects on prolactin and growth hormone in the cultured tumor cells, bromocriptine inhibited both hormones. Thyrotropin-releasing hormone stimulated release of prolactin and growth hormone only slightly (difference not statistically significant). A slight suppressive response to somatostatin was observed for prolactin only. Lamberts et al.[2] have demonstrated 39% inhibition of growth hormone by somatostatin in 12 of 15 pituitary tumor cell cultures from acromegalic subjects.

Several factors, such as a difference in dose, absorption, metabolic clearance, and duration of testing, may explain the discrepancy between the absence of a growth hormone response to stimuli in vivo and the presence of a selective response in vitro.

REFERENCES

1. Tang LKL, Spies GH. Effect of synthetic LH-releasing factor (LRF) on LH secretion in monolayer cultures of the anterior pituitary cells of cynomolgus monkeys. Endocrinology 1974;94:1016.
2. Lamberts SWJ, Verleun T, Oosterom R. The interrelationship between the effects of somatostatin and human pancreatic growth hormone-releasing factor on growth hormone release by cultured pituitary tumor cells from patients with acromegaly. J Clin Endocrinol Metab 1984;58:250.

Local cerebral glucose utilization in fetal sheep exposed to noise

R. M. Abrams, Ph.D., A. A. Hutchison, M.B., Ch.B., K. J. Gerhardt, Ph.D., S. L. Evans, B.S., and J. Pendergast, Ph.D.
Gainesville, Florida

Rate of local cerebral glucose utilization in fetal sheep was measured in conditions of normal laboratory ambient sound (65 to 70 dB sound pressure levels) and broadband noise (105 and 120 dB sound pressure levels) delivered by two earphones situated on the abdominal surface of the ewe. Broadband noise had neither a visible effect on the ewe nor specific effects on the fetal arterial pH, Pa_{O_2}, Pa_{CO_2}, and plasma glucose concentration. However, glucose utilization of many cerebral structures was elevated in noise-exposed fetuses. (AM J OBSTET GYNECOL 1987;157:456-60.)

Key words: Fetus, sound, brain, glucose utilization

The effect of environmental sound on the human fetus in utero is of widespread interest. Developmental biologists speculate that prenatal auditory experiences have subtle but far-reaching implications in human postnatal speech recognition and behavioral maturation.[1] Conversely, among public health specialists there is concern about the possible deleterious effects on fetuses from noise in the workplace.[2] Broadband noise applied to the anterior abdominal wall of pregnant women can result in increased fetal heart rate,[3] blink-startle reflexes,[4] and fetal movements that persist beyond the period of sound stimulation.[5]

In an earlier study we measured the energy metabolism of the fetal sheep brain in normal laboratory sound conditions.[6] Of all the cerebral structures examined, the brain stem auditory nuclei and pathways had some of the highest rates of glucose utilization. We inferred from these clinical and animal findings above that sound is likely to be a major sensory input for the fetus.

The link between cerebral activity evoked by a specific sensory input and the metabolic rate of the specific structures and pathways involved in processing the stimulus is well understood in animals postnatally.[7] Apart from one study[8] of carbon 14–labeled deoxyglucose in central auditory structures in gerbils at various times after birth, there is little known about the metabolic correlates of function in these structures during their ontogeny.

In the present study we measured fetal local cerebral metabolism during the application of intense broadband noise applied by earphones to the shaved abdomen of the pregnant ewe. The aim was to determine if the metabolism in the central auditory pathway was increased above its already high level. An unexpected finding was the metabolic activation of some nonauditory structures, suggesting a diffuse activation of the developing brain by acoustic stimuli.

Methods

Fifteen pregnant, grade Western ewes at 130 to 135 days of gestation were anesthetized with halothane. Catheters were placed in the fetal brachiocephalic artery and superior vena cava and in the maternal iliac artery and inferior vena cava as previously described.[7] Ewes were cart trained during the daily removal and reinstillation of heparin (500 U/ml) in the catheters.

On the day of the experiment to measure local cerebral glucose utilization in fetuses, an experimental ewe and a companion ewe were placed in carts and wheeled to an adjacent empty room. Each of the eight sound-exposed animals was fitted with a 3-inch-wide folded sheet encircling the abdomen and flanks. The sheet contained two TDH-39 earphones (Royal Industries, Phoenix, Arizona) spaced 6 inches on either side of the ventral midline at the level of the anterior aspect of the thigh. Earphones and amplifiers were calibrated with a standard NBS 9A coupler and B & K pressure microphone (Bruel & Kjaer, Marlborough, Massachusetts) to produce broadband noise at inten-

From the Departments of Obstetrics and Gynecology, and Pediatrics, College of Medicine, and the Department of Speech and Statistics, College of Liberal Arts and Sciences, University of Florida.
Aided by Reproductive Hazards in the Workplace Research Grant No. 15-76 from the March of Dimes Birth Defects Foundation and by research Grant HD20084-01, National Institutes of Health.
Received for publication July 14, 1986; revised February 9, 1987; accepted March 6, 1987.
Reprint requests: R. M. Abrams, Ph.D., Department of Obstetrics and Gynecology, University of Florida College of Medicine, JHMHC J-294, Gainesville, FL 32610.

sities of 105 and 120 dB sound pressure level (referenced to 20 μPa). The spectrum was flat to 6000 Hz and began to roll off at approximately 26 dB per octave above that frequency. The sound-exposed animals and seven unfitted control animals stood quietly during a 2-hour preexposure period.

Control samples of fetal and maternal arterial blood were drawn for analyses of glucose concentrations, background carbon 14 levels, P_{O_2}, P_{CO_2}, pH, and hematocrit. Earphones were then activated. Five minutes later, at time zero, 200 to 300 μCi of 2-deoxy-D-[^{14}C]glucose ([^{14}C]DG), specific activity 50 to 56 μCi/mol (American Radiolabeled Chemicals Inc., St. Louis, Missouri), in 2 to 3 ml of physiologic saline solution, warmed to body temperature, was injected into the fetal venous catheter (dead space, 0.6 ml) over 15 to 30 seconds. The injection was followed by a 2 ml flush of saline solution over 10 to 20 seconds. Sampling from the fetal arterial catheter was begun at time zero. Usually five samples of 0.6 ml each were taken during the first minute to characterize the plasma [^{14}C]DG levels that increased and decreased rapidly during and after infusion. Approximately eight additional blood samples were taken at increasing intervals for 40 minutes, after which time the sound was turned off and both fetus and ewe were deeply anesthetized with barbiturate and then killed with an intravenous injection of saturated potassium chloride. The deep vaginal temperature of the mother was measured immediately.

The fetal brain was removed as quickly as possible, frozen in Freon XII at −50° C, and stored at −80° C. Plasma glucose concentration and plasma carbon 14 levels were measured according to published procedures.[6] A computer program (provided by Drs. L. Sokoloff and C. Kennedy, National Institute of Mental Health) was used to calculate the final tissue [^{14}C]DG and the final integrated tissue-specific activity for both gray and white matter.

Brains were sectioned at 20 μm in a Bright cryostat (Bright Instrument Company, Limited, Huntingdon, Combs, England). The distance advanced during a cutting cycle was verified by micrometry. Three consecutive sections out of 28 were mounted on coverslips, dried immediately, and exposed along with National Institute of Mental Health standardized, carbon 14 incorporated plastic discs (Amersham Corp., Arlington Heights, Illinois) to Kodak SB-5 film for 7 days. The density of brain structure and discs was determined with a densitometer equipped with a 0.5 mm aperture (Sargent-Welch Scientific Co., Skokie, Illinois).

Homogeneity of densities within many structures, for example, in the hypothalamus, mamillary body, caudate, putamen, superior colliculus, lateral geniculate, reticular formation, optic chiasm, and corona radiata, required only bilateral readings in three to four sections to obtain a representative average value. Structures with marked heterogeneity of densities, that is, the hippocampus and vermis, were surveyed in a random fashion to obtain an unweighted average of values. In smaller heterogeneous structures, including the brain stem nuclei, the average of single readings of highest density in each of six to eight sections was recorded.

A calculation of tissue content of [^{14}C]DG was performed with National Institute of Mental Health calibrated Amersham plastic standards. The lumped constant used, 0.401, was determined in earlier experiments in fetal and newborn sheep.[6] Local cerebral glucose utilization in micromoles per 100 gm per minute was calculated according to the formula published by Sokoloff et al.[7]

The Wilcoxon rank sum test for unpaired data was used to compare data collected on control fetuses with data on sound-treated fetuses ($p < 0.05$). For the purposes of this test, no distinction was made between those fetuses receiving 105 dB and those receiving 120 dB sound pressure levels.

Results

Two animals from sound stimulation experiments were not included in the statistical analysis of local cerebral glucose utilization. Distended cerebral ventricles were found in one fetus during the sectioning procedure. No explanation or cause for this hydrocephalus could be found. In a second fetus, intermittent, position-related difficulties in drawing blood from the arterial catheter necessitated vigorous palpation of the abdomen of the ewe to force movement of the fetus during the sampling procedure. The possibility that vestibular or tactile stimulation might have altered local cerebral glucose utilization was considered. In both cases, however, local cerebral glucose utilization was within the ranges found in the remaining six sound-stimulated fetuses.

The gestational ages, fetal weights, number of days after operation, fetal blood gas determinations, fetal plasma glucose levels, and maternal temperature in the experimental and control groups were not significantly different (Table I).

In all structures evaluated mean local cerebral glucose utilization was higher in sound-stimulated animals as a group than in control animals (Table II). However, the significance of these differences was variable because of considerable overlapping in local cerebral glucose utilization values. Of the brain stem auditory nuclei, only the cochlear nuclei showed a significant sound-induced rise in glucose utilization. Other structures exhibiting a rise that reached significance ($p < 0.05$) were vestibular nuclei, inferior olives, habenula,

Table I. Gestational ages, weights, days after operation, blood gas and pH levels, and arterial plasma glucose concentrations of control and sound-exposed fetal sheep in utero and maternal vaginal temperatures at termination of experiment to measure local cerebral glucose utilization

	Control fetuses						
Fetus	*149*	*W-73*	*W-75*	*W-125*	*158*	*W-95*	*W-84*
Gestational age (days)	135	136	136	137	138	140	141
Fetal weight (kg)	2.1	3.1	3.20	3.1	2.5	4.5	3.55
Days after operation	5	4	5	3	6	7	7
Fetal arterial							
pH	7.34	7.36	7.39	7.37	7.38	7.35	7.37
P_{CO_2}	55.8	55.9	55.5	54.3	47.4	60.4	49.2
P_{O_2}	21.8	21.5	23.4	20.8	20.9	22.9	24.4
Fetal arterial plasma glucose (mg/ml)	0.18	0.18	0.17	0.18	0.18	0.28	0.20
Maternal temperature (° C)	39.0	39.6	39.0	39.5	39.6	39.4	—
Sound pressure level (dB)	55-60	55-60	55-60	55-60	55-60	55-60	55-60

Table II. Local cerebral glucose utilization (micromoles/100 gm/min) in control fetuses and in sound-exposed fetuses

Structure	*Control fetuses (n = 7)*	*Sound-exposed fetuses (n = 6)*	*p Value*
Motor/sensory cortex	18 ± 2.6	26 ± 3.2	0.1302
Visual cortex	18 ± 3.0	27 ± 3.4	0.1584
Auditory cortex	18 ± 2.6	25 ± 3.3	0.3044
Cingulate gyrus	23 ± 3.5	34 ± 4.4	0.1113
Thalamus	23 ± 3.2	37 ± 4.3	0.0504
Medial geniculate	31 ± 3.7	46 ± 4.6	0.0683
Lateral geniculate	35 ± 5.6	43 ± 5.9	0.2790
Hypothalamus	15 ± 1.9	26 ± 6.4	0.2464
Mamillary body	36 ± 4.8	51 ± 10.2	0.3508
Hippocampus	23 ± 2.8	36 ± 5.7	0.0876
Amygdala	21 ± 2.6	34 ± 9.8	0.4340
Septum	20 ± 2.7	34 ± 6.9	0.1298
Caudate	17 ± 2.5	28 ± 4.2	0.0746
Putamen	19 ± 2.8	28 ± 2.4	0.0664
Subthalamic nuclei	35 ± 5.0	57 ± 9.5	0.0753
Vestibular nuclei	33 ± 3.6	58 ± 9.9	0.0467
Cochlear nuclei	26 ± 3.7	49 ± 4.7	0.0215
Superior olives	42 ± 5.8	61 ± 10.8	0.2790
Inferior olives	31 ± 3.5	52 ± 6.4	0.0360
Inferior colliculus	59 ± 7.3	91 ± 8.5	0.0876
Superior colliculus (stratum superficiale)	26 ± 5.0	43 ± 6.0	0.1254
Superior colliculus (stratum profundum)	16 ± 2.7	25 ± 2.8	0.1254
Habenula	22 ± 2.4	40 ± 5.2	0.0185
Preoptic area	15 ± 2.2	23 ± 4.2	0.1831
Spinal cord, ventral horns	18 ± 2.5	27 ± 2.8	0.0680
Reticular formation	20 ± 3.8	26 ± 3.1	0.1986
Lateral lemniscus	33 ± 6.2	49 ± 5.6	0.0978
Periaqueductal gray	13 ± 2.3	20 ± 2.6	0.1406
Nuclei of oculomotor nerves	24 ± 3.3	37 ± 2.8	0.0314
Red nuclei	23 ± 2.6	38 ± 4.2	0.0279
Vermis	18 ± 2.5	28 ± 2.1	0.0673
Cerebellar cortex	13 ± 1.9	21 ± 2.8	0.0530
Cerebellar nuclei	26 ± 2.9	46 ± 6.0	0.0467
Flocculus	22 ± 3.1	36 ± 3.3	0.0277
Spinal cord, lateral columns	11 ± 1.6	17 ± 1.7	0.0978
Internal capsule	12 ± 2.0	20 ± 0.9	0.0573
Corona rdiata	6 ± 1.1	10 ± 1.1	0.0767
Optic chiasm	12 ± 2.0	14 ± 2.0	0.5264

Wilcoxon rank sum test for unpaired data; mean and SEM.

Sound-exposed fetuses					
124	*108*	*105*	*W-62*	*W-86*	*W-72*
137	136	135	135	133	136
2.25	2.85	3.65	2.85	3.55	3.80
2	2	2	6	6	7
7.33	7.37	7.31	7.35	7.36	7.38
49.3	52.5	55.6	52.5	51.6	54.2
14.4	26.6	27.3	24.3	22.8	26.2
0.18	0.24	0.17	0.21	0.37	0.17
39.5	39.7	38.9	—	39.6	39.4
105	105	105	120	120	120

nuclei of the oculomotor nerves, red nuclei, cerebellar nuclei, and flocculus. Other major auditory structures evaluated were auditory cortex ($p < 0.30$), medial geniculate ($p < 0.07$), superior olives ($p < 0.28$), inferior colliculus ($p < 0.09$), and lateral lemniscus ($p < 0.10$).

Comment

The effects on the human fetus of environmental sound are well-known. Thus sound-induced fetal movements[5, 9] and fetal cardioacceleration[10, 11] usually, but not always, follow an intense pure tone or broadband of tones applied to the anterior abdominal wall. Generally, the sound source is applied directly over the fetal head as palpated or seen in ultrasound scans.

To understand more fully the importance of sound in human prenatal development, we have used the fetal sheep as a model. First, we measured the metabolic rate of the fetal central auditory structures in normal laboratory sound conditions.[6] Glucose utilization in these structures was higher than most structures in the fetal sheep brain. Rates of utilization in the cochlear nucleus, superior olives, lateral lemniscus, and inferior colliculus rose rapidly near the beginning of the third trimester and remained high until term, after which there was a 50% increase. In contrast, utilization of glucose in the brain as a whole rose more linearly throughout the last third of gestation.

In subsequent experiments[12] we documented a significant decrease in local cerebral glucose utilization during administration of ketamine and thiopental to induce anesthesia. Also we characterized the sound environment of the fetus[13] and confirmed that it was richer and more varied than previously thought.[14, 15] In fact, sounds with frequencies below 2 kHz were attenuated very little.[13-15]

In the present study we attempted to determine if sound presented to the surface of the abdomen could produce any increase in central auditory metabolic rate, from which we could infer that neuronal activity along this pathway was enhanced. There is ample documentation that sound results in a significant rise in glucose utilization in brain stem nuclei subserving the auditory system,[16-18] but there was no mention in the literature reviewed of an enhancement in the nonauditory structures.

The reason many fetal gray and white matter structures, which are nonspecific for the auditory system, appeared to be affected by sound is not readily understood, but functional activity in specific pathways may not be a prominent feature during the early stages of development. Myelinization proceeds in a rostral direction; before its completion, auditory stimulation may result in a nonspecific, diffuse stimulation of metabolic activity in many brain structures. This widespread, sound-induced requirement for free energy may be vital for normal growth and development of the brain.

The earphones used in this study produced very high intensity broadband noise but over a relatively small area. If the fetal head were not directly in this field and close to the sound source, some attenuation of sound would be expected. The abdomen and pregnant uterus are not acoustically uniform. Also to be considered as a source of the large variability in the local cerebral glucose utilization results would be the predominant behavioral state during the time that local cerebral glucose utilization was measured. Both fetal brain oxygen consumption[19] and glucose utilization[20] are increased during low-voltage electrocortical activity. A clearer picture of the specific and general effects of sound as a useful test for fetal neuronal function should emerge if both the position of the fetal head and the fetal behavioral state are known.

We gratefully acknowledge the advice and assistance of Drs. C. Kennedy, C. Oliver, J. Hicks, and K. Bzoch, and Messrs. R. Trustee and R. Cooper.

REFERENCES

1. Kolata G. Studying learning in the womb. Science 1984;225:302.
2. Report of Working Group 85, Committee on Hearing, Bioacoustics, and Biomechanics, Assembly of Behavioral and Social Scientists, National Research Council. Prenatal effects of exposure to high-level noise. Washington DC: National Academy Press, 1982.
3. Grimwade JC, Walker DW, Wood C. Sensory stimulation of the human fetus. Aust J Ment Retard 1970;2:63.
4. Birnholz JC, Benacerraf BR. The development of fetal hearing. Science 1983;222:516.
5. Gelman SR, Wood S, Spellacy WN, Abrams RM. Fetal movements in response to sound stimulation. AM J OBSTET GYNECOL 1982;143:484.
6. Abrams RM, Ito M, Frisinger JE, Parlak CS, Pettigrew KD, Kennedy C. Local cerebral glucose utilization in fetal and neonatal sheep. Am J Physiol 1984;246:R608.
7. Sokoloff L, Reivich M, Kennedy C, et al. The [^{14}C]-deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure and normal values in the conscious and anesthetized albino rat. J Neurochem 1977;28:897.
8. Ryan AF, Woolf NK, Sharp FR. Functional ontogeny in the central auditory pathway of the mongolian gerbil. Exp Brain Res 1982;47:428.
9. Bernard J, Sontag LW. Fetal reactivity to tonal stimulation: a preliminary report. J Genet Psychol 1947;70:205.
10. Serafini P, Lindsay MBJ, Nagey DA, Pupkin MJ, Tseng P, Crenshaw C. Antepartum fetal heart rate response to sound stimulation: the acoustic stimulation test. AM J OBSTET GYNECOL 1984;148:41.
11. Goodlin RC, Schmidt W. Human fetal arousal levels as indicated by heart rate recordings. AM J OBSTET GYNECOL 1972;114:613.
12. Abrams RM, Cooper R. Effect of ketamine on local cerebral glucose utilization in fetal sheep. AM J OBSTET GYNECOL 1987;156:1018.
13. Gerhardt K, Abrams RM. The sound environment of the fetal sheep in utero. Fed Proc 1987;46:356.
14. Armitage SE, Baldwin BA, Vince MA. The fetal sound environment of sheep. Science 1980;208:1173.
15. Vince MA, Billing AE, Baldwin BA, Toner JN, Weller C. Maternal vocalisations and other sounds in the fetal lamb's sound environment. Early Hum Dev 1985;11:179.
16. Webster WR, Serviere J, Batini C, Laplant S. Autoradiographic demonstration with 2-[^{14}C]deoxyglucose of frequency selectivity in the auditory system of rats under conditions of functional activity. Neurosci Lett 1978; 10:43.
17. Sharp FR, Ryan AF, Goodwin P, Woolf NK. Increasing intensities of wideband noise increase [^{14}C]2-deoxyglucose uptake in gerbil central auditory structures. Brain Res 1981;230:87.
18. Canlon B, Schacht J. Acoustic stimulation alters deoxyglucose uptake in the mouse cochlea and inferior colliculus. Hearing Res 1983;10:217.
19. Richardson BS, Patrick JE, Abduljabbar H. Cerebral oxidative metabolism in the fetal lamb: relationship to electrocortical state. AM J OBSTET GYNECOL 1985;153:426.
20. Abrams RM, Hutchison AA, Jay TM, Kennedy C. Cerebral glucose utilization increased during rapid eye movement sleep. Ann Neurol 1986;20:401.

Transplacental stimulation of functional and morphologic fetal rabbit lung maturation: Effect of thyrotropin-releasing hormone

Uday Devaskar, M.D., Kenneth Nitta, M.D., Karl Szewczyk, M.D., H. Farouk Sadiq, M.D., and Daphne deMello, M.D.

St. Louis, Missouri

We investigated the effect of maternally administered thyrotropin-releasing hormone on functional and morphologic fetal lung maturation. Thyrotropin-releasing hormone (40 μg/kg/day) or the vehicle was injected intravenously into the New Zealand White rabbit does on days 25, 26, and 27 of gestation. On day 27 of pregnancy, the does were killed and the fetuses were delivered. The functional pulmonary maturity was assessed by pressure-volume hysteresis while morphologic maturity was assessed by histologic techniques. Enhanced functional and morphologic fetal lung maturation was noted in animals treated with thyrotropin-releasing hormone when compared with controls. An important role of thyrotropin-releasing hormone in fetal lung maturation is proposed. (AM J OBSTET GYNECOL 1987;157:460-4.)

Key words: Thyrotropin-releasing hormone, fetus, lung maturation, hyaline membrane disease, placenta

From the Departments of Pediatrics and Pathology, St. Louis University School of Medicine and The Pediatric Research Institute.
This work was supported by a grant from the Fleur de Lis Foundation.
Received for publication November 11, 1986; accepted March 12, 1987.
Reprint requests: Uday P. Devaskar, M.D., Division of Neonatology, Cardinal Glennon Children's Hospital, 1465 So. Grand Blvd., St. Louis, MO 63104.

Mammalian fetal lung development can be influenced by several hormones including corticosteroid, insulin, androgens, estrogens, catecholamines, epidermal growth factor, prolactin, thyroid hormones, and thyrotropin-releasing hormone (TRH).[1] Glucocorticoids administered antenatally to women in premature

labor reduce the risk of hyaline membrane disease in premature neonates.[1] However, the beneficial effect of corticoids may be dependent on time, fetal sex, and gestational age.[1] Corticosteroids are relatively contraindicated when pregnancy is complicated by placental insufficiency, premature rupture of the amniotic membranes, maternal infection, or diabetes.[2] All of these situations are frequently encountered clinically.

Administration of TRH into the rabbit doe results in an increase in total phospholipid and phosphatidylcholine in the fetal lung lavage, while the phosphatidylcholine/sphingomyelin ratio increases from 1.0 to 2.2.[3] These changes in the fetal lung lavage phospholipid content and composition are suggestive of increased lung maturation. Although the clinical use of TRH, alone or in combination with corticosteroids, has been suggested for the prevention of hyaline membrane disease,[3] the mechanisms of the effects of TRH, its influence on other parameters of fetal lung development, and the possible side effects remain unclear. Rooney et al.[3] postulated that the administration of TRH to the rabbit doe lends to its transfer across the placenta, which in turn stimulates fetal thyroid activity.[3] However, the demonstration of placental transfer of TRH or the quantitation of fetal plasma thyroid hormones was not performed.[3] In this study we investigated whether administration of TRH to the rabbit doe results in its passage across the placenta. In addition, we investigated the effect of maternal administration of TRH on functional and morphologic fetal lung development, fetal lung glycogen content, and maternal and fetal plasma total triiodothyronine and free thyroxine concentration. From the results we conclude that TRH crosses the rabbit placenta from the mother to the fetus and that functional and morphologic fetal lung development can be accelerated transplacentally by maternal administration of TRH.

Material and methods

Animal care and euthanasia. Pregnant New Zealand White rabbits of known gestation time (0 = time of breeding; term = ~31 days) were purchased from a local supplier, housed individually, and allowed to drink water and eat Rabbit Chow (Purina, St. Louis, Missouri) as desired. To investigate whether TRH crosses the placenta, we injected 10 μCi of iodine-125–labeled TRH [(Pyro)Glu-His-Pro-NH2, molecular weight 486.3, specific activity 2200 Ci/mmol, New England Nuclear, Boston, Massachusetts] per doe (n = 3) on day 27 of gestation. Maternal arterial blood was collected 10 minutes before and 1, 5, 10, 20, 30, 60, and 90 minutes after the intravenous injection of ^{125}I-labeled TRH. The does were killed with intravenous pentobarbital (100 mg/kg) and the fetal blood was collected after decapitation.[5] The radioactivity in 400 μl of the plasma was counted in a gamma radiation counter (Packard Instruments, Downers Grove, Illinois) for 2 minutes. The degradation of ^{125}I-labeled TRH was assessed by 50% trichloroacetic acid precipitation.

To investigate the effect of maternal administration of TRH (L-pyroglutamyl-L-histidyl-L-prolinamide, molecular weight 362.4, Sigma Chemical Co., St. Louis, Missouri) on fetal lung development, TRH (40 μg/kg/dose) or an equivalent volume of normal saline solution was injected intravenously between 8 and 9 AM on days 25, 26, and 27 of pregnancy. Forty-five minutes after the third dose, the does were killed by intravenous pentobarbital. Immediately before the animals were put to death, maternal blood was collected from the ear artery. The uterus was exposed after laparotomy. The three to four fetuses on the extreme right of the uterus were killed with 10 mg each of intramuscular pentobarbital through the uterine wall. The rest of the fetuses were delivered and immediately decapitated. Free-flowing blood was collected for the measurement of total triiodothyronine and free thyroxine.[5] A reliable assay for plasma TSH and total thyroxine in rabbit plasma was not available. Lungs from the decapitated fetuses were collected for the quantitation of glycogen.[5] Pentobarbital-injected fetuses were delivered with the amniotic sac and placenta intact and used for the pressure-volume hysteresis.

Pressure-volume curve. The method of performing dynamic pressure volume curve experiments on the closed chest rabbit fetuses has been described.[6] The blunt needle of a 19-gauge butterfly needle was inserted into the trachea and fixed in place with silk. The other end of the butterfly needle was attached to a three-way stopcock that was connected to a manometer and to a 10 ml graduated syringe via thin plastic tubing. The syringe was mounted on a Harvard pump (Harvard Apparatus Co., South Natic, Massachusetts) that is capable of infusing and withdrawing. The lungs were gradually inflated with air to 40 cm H_2O and then deflated slowly to 0 cm H_2O. The lungs were deflated to −30 cm H_2O to remove residual air and then equilibrated to 0 cm H_2O. The lungs were again inflated to 40 cm H_2O. After equilibration at an inflation pressure of 40 cm H_2O, the lungs were gradually deflated to 0 cm H_2O. The pressure on the manometer was recorded after each 0.4 ml of air was withdrawn. The total lung capacity (that is, the amount of air injected in the lung during the second inflation) and the stability of the lung during the second deflation at 0, 5, 10, 20, 35, and 40 cm H_2O were derived. After the pressure-volume experiment the lungs were dissected, gently blotted to remove excess fluid, and weighed. These lungs were fixed at atmospheric pressure by immersion in 10% paraformaldehyde.

Histologic evaluation of the fetal lung. Sagittal sections from the right upper lobe of the fixed whole lung

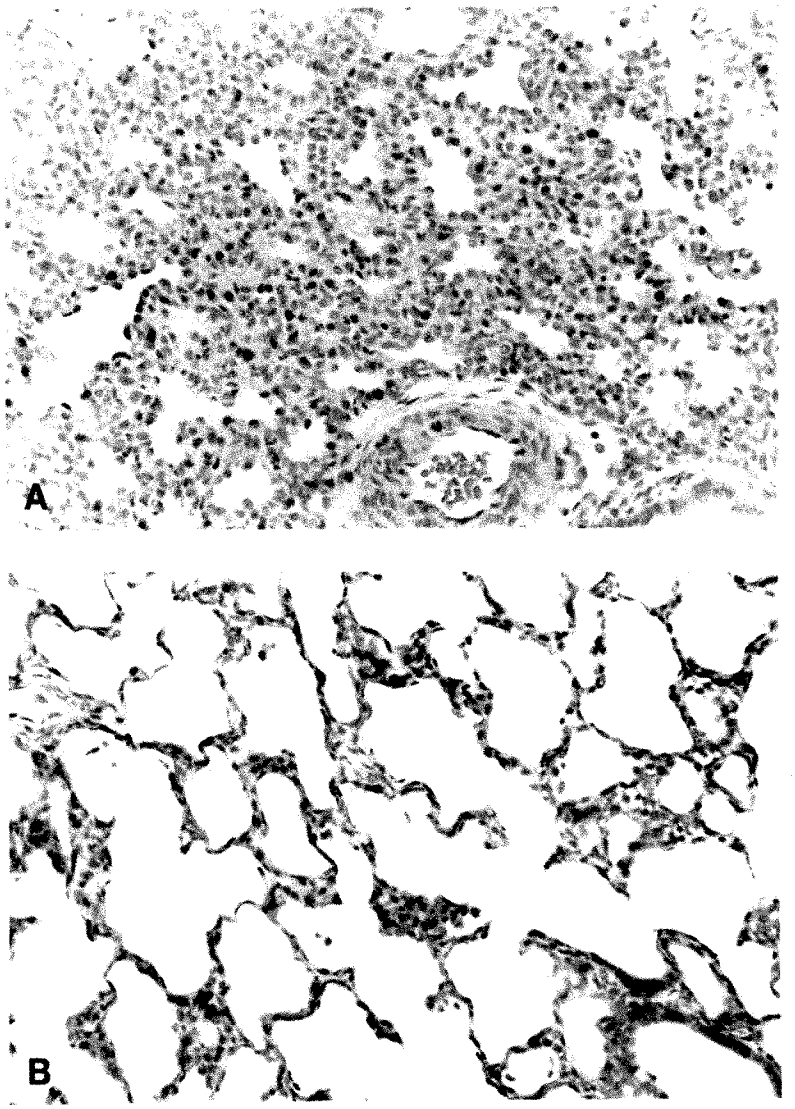


Fig. 1. Morphologic features of control *(A)* and TRH-treated *(B)* fetal lungs.

Table I. Fetal weight and lung glycogen and plasma thyroid hormone concentrations in control and TRH-treated animals

	Control (n = 11)	*TRH-treated (n = 10)*
Fetal weight (gm)	28.5 ± 0.6	27.8 ± 0.7
Fetal lung glycogen (μmol of glycosyl units/gm)	31 ± 3	30 ± 4
Plasma triiodothyronine (ng/ml)		
Maternal	2.2 ± 0.2	1.8 ± 0.2
Fetal	1.0 ± 0.1	1.0 ± 0.1
Plasma free thyroxine (ng/dl)		
Maternal	0.7 ± 0.1	0.5 ± 0.2
Fetal	0.08 ± 0.001	0.06 ± 0.05

Numbers in parentheses represent the number of does in each group.

were embedded in Parafilm, and 6 μm sections from each block were stained with hematoxylin and eosin. In addition to conventional microscopic examination, lung volume proportions were analyzed with an integrating eyepiece of the point-counting type, as per the principles outlined by Chalkley.[7] The relative volume (V) of alveolar lumen (al) and the interalveolar septa (ias) was determined.[7] The alveolar expansion index (Ia) was calculated as follows:

$$Ia = \frac{Val}{Vias}$$

Statistical analysis

All data are mean ± SEM. Statistical significance was determined by Student's two-tailed *t* test. If pneumo-

Table II. Lung/body weight and the stability of fetal lungs during deflation in control and TRH-treated animals

Group	Lung/body weight	Total lung capacity (ml/gm)	Air retained in lung during deflation (% of total lung capacity)					
			0 cm H_2O	5 cm H_2O	10 cm H_2O	20 cm H_2O	35 cm H_2O	40 cm H_2O
Control (n = 30)	0.029 ± 0.007	1.45 ± 0.1	2.0 ± 0.04	15 ± 2	33 ± 2	80 ± 1	100	100
TRH-treated (n = 24)	0.031 ± 0.008	1.43 ± 0.2	19 ± 2*	37 ± 3*	58 ± 3*	87 ± 1*	100	100

Numbers in parentheses represent the number of fetuses in which successful pressure-volume hysteresis was performed.
*$p < 0.05$.

thorax developed at any time before completion of the second deflation, that experiment was excluded from statistical analysis. Numbers in parentheses in Table I represent the number of does in which vehicle or TRH was administered. Numbers in parentheses in Table II represent the number of successful pressure-volume experiments.

Results

The trichloroacetic acid precipitability of radioactivity in fresh ^{125}I-labeled TRH was ~60%. The TCA precipitability of radioactivity in the maternal and fetal plasma after 90 minutes of ^{125}I-labeled TRH injection was also ~60% in all three experiments. These results imply that there was no degradation of ^{125}I-labeled TRH during this period. The amounts of radioactivity (counts per minute per 0.4 ml) in the maternal plasma were 100 ± 5, 12,000 ± 3000, 10,000 ± 2000, 8000 ± 1500, 6000 ± 1000, 4800 ± 600, 2600 ± 300, and 1800 ± 150, respectively, at 10 minutes before and 1, 5, 10, 20, 30, 60, and 90 minutes after an intravenous bolus of 100 μCi of ^{125}I-labeled TRH. The mean radioactivity in 0.4 ml of fetal plasma in all treated animals was higher (1800 ± 100 cpm) than the control (90 ± 5, n = 3, $p < 0.001$). It can be concluded that TRH crosses the rabbit placenta from the mother to the fetus.

There were no obvious undesirable effects in the mother or the fetuses after TRH treatment. None of the TRH-treated or control does delivered prematurely. All does in control and TRH groups gained 200 ± 6 gm (n = 11) and 195 ± 8 gm (n = 10), respectively, from 25 to 27 days of gestation. Fetal weight, fetal lung glycogen, and fetal and maternal plasma triiodothyronine and free thyroxine concentrations were similar in control and TRH-treated animals (Table I).

The lung–to–body weight ratio and the total lung capacity among control and TRH group fetuses were not significantly different (Table II). The stability of the fetal lungs during deflation at 0, 5, 10, and 20 cm H_2O pressure in TRH-treated animals was higher than that in the control group (Table II, $p < 0.05$).

On histologic examination a clear difference between TRH-treated and control fetal lungs was noted (Fig. 1). Alveolar air expansion was more pronounced in TRH-treated fetuses. The alveolar expansion index in control fetuses (0.61 ± 0.032, n = 48) was less than that in TRH-treated fetuses (0.77 ± 0.069, n = 48) ($p < 0.05$).

Comment

Controversy has been expressed as to whether TRH can cross all mammalian placentas.[8] TRH crosses the placenta of the rat, rhesus monkey, and human.[8] In contrast to these studies, a report claimed lack of placental transport of TRH in sheep.[9] In the present study we have shown that TRH crosses the placenta from the mother to the fetus in the rabbit. The difference in the placental transport of TRH among these mammalian species may be due to the different structures of the placenta. While the human, monkey, or rat placenta is hemochorial, the sheep placenta is epitheliochorial.[10] The rabbit placenta can be hemochorial or hemoendothelial.[10]

Fetuses from TRH-treated does demonstrated enhanced functional and morphologic lung maturation. These results further support the hypothesis that TRH may be important in fetal lung development.[3] Increased stability of lungs on deflation in TRH-treated fetuses may be due to increased availability of alveolar surfactant, changes in connective tissue elasticity, or the degree of alveolation or a combination of these factors. Pressure-volume experiments with saline solution infusion would have been helpful but were technically difficult. Since TRH administration has been shown to enhance secretion of pulmonary surfactant in the fetal rabbit at a similar gestational age,[3] it is likely that the stability of the lungs on deflation in TRH-treated fetuses in the present study was due to increased alveolar surfactant.

Maternal and fetal plasma thyroid hormone concentrations in TRH-treated animals were similar to control values. It can be concluded that an increase in maternal

or fetal plasma thyroid hormone does not occur within 45 minutes of TRH administration in rabbit. Although no data are available in the rabbit, an increase in maternal or umbilical cord plasma thyroid hormone concentration after maternal administration of TRH did not occur until 3 to 4 hours in the human.[11] It is possible that maternal and/or fetal plasma thyroid hormone concentrations did increase after the first two doses of TRH on days 25 and 26 of pregnancy, but this possible surge in the plasma thyroid hormone concentration was not sustained enough to be seen on day 27. On the other hand, it is conceivable that enhanced fetal lung maturation from TRH stimulation may occur without a significant stimulation of fetal thyroid hormone activity. Surfactant production and/or release may be under neurohumoral control.[1] TRH has been shown to serve as a neurotransmitter and therefore may act independently of the fetal pituitary-thyroid axis.[3] The human placenta produces TRH and TRH has been demonstrated in both maternal and fetal plasma.[12, 13] Maternal plasma concentrations of TRH increase during pregnancy and TRH administered to the pregnant woman stimulates the fetal pituitary-thyroid axis.[11, 14] Although the physiologic significance of these observations remains unclear, it is possible that TRH may be important in the maturation of the human fetal lung.

Acceleration of fetal lung maturation after TRH therapy has not yet been determined to improve respiratory function and enhance survival after premature delivery. Whether simultaneous administration of TRH and corticosteroids does indeed have an additive effect in enhancing fetal lung maturation remains to be examined. Future animal experimentation seems necessary before a clinical trial of TRH, alone or in combination with corticosteroids, is initiated.

We thank Dr. William J. Keenan for review of the manuscript.

REFERENCES

1. Rooney SA. Am Rev Respir Dis 1985;181:439-60.
2. Hallman M, Gluck L. The newborn. Pediatr Clin North Am 1982;1057:29.
3. Rooney SA, Marino PA, Gobran LI, Gross I, Warshaw JW. Pediatr Res 1979;13:623-5.
4. Avery ME, Aylward G, Creasy R, Little B, Stripp B. Am J Obstet Gynecol 1986;155:2-5.
5. Devaskar UP, Chechani V, Sadiq HF, Devaskar S. Dev Pharmacol Ther 1986;9:115-23.
6. Kotas RV, Avery ME. J Appl Physiol 1971;39:353.
7. Chalkley HW. JNCI 1941;4:47-9.
8. Azykizawa M, Murata Y, Ikenoue T, Martin CB Jr, Harshman JH. J Clin Endocrinol Metab 1976;43:1020-8.
9. Thomas AL, Jack PMB, Mannus JG, Nathanielsz PW. Biol Neonate 1975;26:109.
10. Metcalfe J, Bartels H, Moll W. Physiol Rev 1967;47:782.
11. Roti E, Gnudi A, Braverman LE, et al. J Clin Endocrinol Metab 1981;53:813.
12. Kaufman JM, Elewaut A, Vermeulan A. Acta Endocrinol 1983;104:320-6.
13. Siler-Khodr TM. Clin Perinatol 1983;10:553-6.
14. Amino N, Yamada T, Mitsuma T, et al. J Clin Endocrinol Metab 1981;53:1288.

The effect of severing the spinal cord of fetal lambs on length of gestation

Romana A. Nowak, Ph.D., Susan E. Samaras, B.S., Mary C. Chorney, M.S., D.V.M., Daniel R. Hagen, Ph.D., and Philip J. Dziuk, Ph.D.
Urbana, Illinois

Lambs with spinal cords that had been severed near the head at 50 days of gestation were born after a shortened gestation of about 128 days. Lambs with severed spinal cords born as a twin with an intact lamb had a normal gestation of 148 days. The presence of an intact spinal cord or the signals that it might carry apparently influence the length of gestation in the ewe. (Am J Obstet Gynecol 1987;157:464-7.)

Key words: Lambs, cordotomy, gestation

From the Department of Animal Sciences, University of Illinois.
Received for publication November 7, 1986; revised March 3, 1987; accepted March 12, 1987.
Reprint requests: Philip Dziuk, Ph.D., University of Illinois, 1301 Taft Drive, Urbana, IL 61801.

The normal fetal hypothalamic-pituitary-adrenal axis has been shown to influence initiation of parturition in sheep. Infusion of adrenocorticotropic hormone (ACTH) but not other pituitary hormones caused pre-

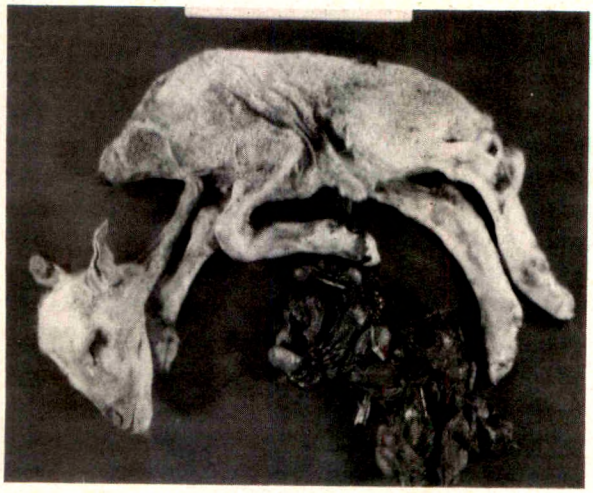


Fig. 1. Lamb born at 145 days of gestation with only a strand of vascular tissue joining the head and body. Separation was done at 45 days of gestation.

mature delivery in 4 to 7 days.[1] Liggins et al.[2] showed that hypophysectomy of fetal lambs resulted in delayed parturition presumably because of absence of secretions from the fetal adrenal cortex. These experiments showed that an intact fetal pituitary-adrenal axis was important for the initiation of parturition in sheep.

How the increases in fetal ACTH and subsequently fetal corticoids are initiated is unclear. The possible role of neurosecretory hormones from the hypothalamus or posterior pituitary gland has not been investigated thoroughly. When the anterior pituitary gland was destroyed or removed in fetal lambs,[2] the posterior pituitary was undoubtedly also removed. Decapitation of a sheep fetus delayed parturition,[3] but in this case the posterior pituitary gland and the brain were also removed. Schriefer et al.[4] observed that the content of oxytocin in pituitary glands from neonatal rats was reduced by parturition, which indicated possible release of oxytocin from the fetal posterior pituitary gland during labor. In addition, administration of an oxytocin antibody to rat fetuses but not to their mothers increased the length of gestation by 18 hours. Oxytocin in the plasma of fetal lambs was significantly higher than in the mother just before parturition.[5] Neurophysins associated with both oxytocin and vasopressin have been described in the fetal sheep pituitary gland.[6] Alexander et al.[7] showed that hypoxia was effective in causing vasopressin release, which was accompanied by an increase in neurophysin and ACTH. It is possible that these neurophysins are the triggering stimuli for oxytocin, vasopressin, and ACTH release before parturition.

In a preliminary experiment we disrupted the normal neural path of fetal lambs by severing the spinal cord in the upper cervical area. Parturition occurred but with varied gestation. Thus the involvement of a neural signal to the fetal hypothalamic-pituitary-adrenal axis may influence initiation of parturition. We performed the following studies in an attempt to understand the involvement.

Table I. Mean length of gestation and weight of intact lambs, lambs with severed spinal cords, or lambs with pinched tracheas

Treatment	*Length of gestation (days)*	*Weight (kg)*
Pinched trachea	149 ± 1.7	3.8 ± 0.3*
Severed spinal cord	128 ± 2.3*	1.86 ± 0.04*
Twins, one intact and one severed	147 ± 0.8	3.2 ± 0.07 (intact) 2.9 ± 0.1 (severed)
Intact control	148 ± 1.6	3.2 ± 0.06

Values are expressed as the mean ± SEM.
*Indicates that value is significantly different from control ($p < 0.05$).

Material and methods

Preliminary study. In the preliminary study an attempt was made to decapitate fetal lambs at about 45 days of gestation in 29 ewes in the same manner as in fetal pigs.[8] Decapitation resulted in fetal death and resorption in all but one case. This one lamb was born after 145 days of gestation. It weighed 1397 gm, was edematous, had no wool, the neck stump was healed, and the intestines contained pastelike material. In several other lambs in which decapitation was attempted, a remnant of vascular tissue connected the head and the body (Fig. 1). These lambs were born after various periods of gestation, ranging from about 100 to 160 days. One lamb with a crushed but intact head weighed 8 kg at 174 days of gestation, when it was removed by hysterotomy. Thus complete decapitation was ordinarily fatal, but disruption of the usual neural pathway was not, provided a vascular connection persisted between the head and the body. To pursue the observations further, a subsequent study was conducted in which the spinal cord was severed in a systematic and uniform manner.

Animals. Forty-six mature crossbred ewes were maintained outdoors under a normal seasonal photoperiod. During the breeding season crossbred rams that wore marking harnesses were introduced into the flock. Ewes were observed daily for marks. Ewes were considered pregnant if they were not marked again. The age of the fetus was verified by palpation at operation.

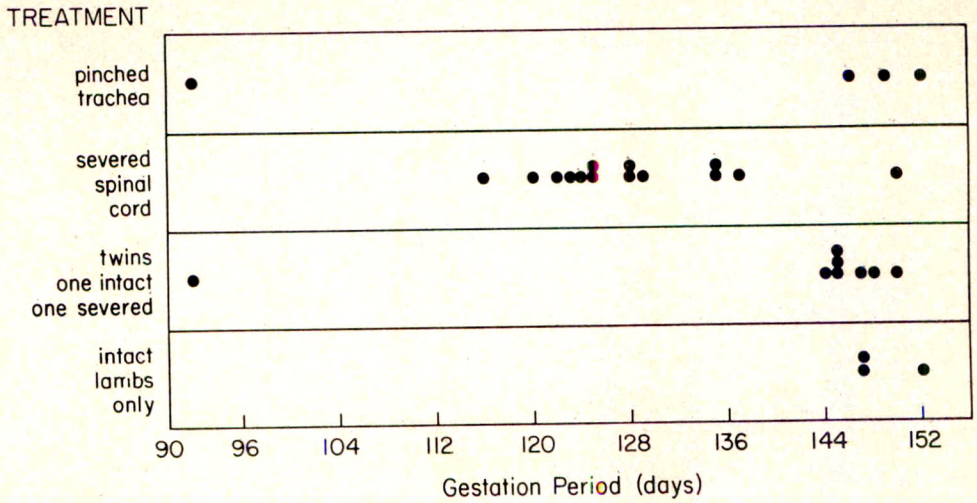


Fig. 2. Gestation for each ewe that contained lambs treated as indicated at 50 to 70 days of gestation.

Animals were cared for and used under the guidelines of the National Institutes of Health for such animals.

Experimental design. Ewes were assigned at 50 to 70 days of gestation to one of four treatment groups: (1) intact control—laparotomy with no surgical manipulation was performed on the fetus; (2) fetal sham operation—the ventral aspect of the neck and trachea of the fetus was pinched and crushed; (3) the spinal cord of the fetus was severed; (4) the spinal cord of one fetus was severed, whereas the twin fetus was left intact.

Anesthesia was induced with an intravenous injection of 17 ml of pentobarbital sodium solution (65 mg/ml, Holmes Serum Co., Springfield, Illinois) and maintained with halothane and a closed-circuit anesthesia apparatus.[9] The ovaries and uterus were exposed through a midventral incision and the number of corpora lutea and fetuses recorded. Spinal cord transections were performed by grasping the fetus through the uterine wall, orienting the fetus by palpation, and then severing the cord with thumb and forefinger in the cervical area. The spinal cord was severed as close to the head as possible, usually between C-1 and C-3. The ventral aspect of the neck and trachea was pinched in a similar manner in the second treatment group. All ewes were observed daily for delivery of lambs.

Lambs were weighed after delivery. The spinal cord was examined grossly to determine the completeness of transection. The lungs were removed and placed in water to determine flotation. The digestive tract was examined for the presence of meconium. Lambs in which the trachea had been pinched were examined to determine the extent of the crushing of the trachea and that the spinal cord was intact.

Statistical analysis. Results were analyzed with Student's t test. Differences were considered significant when $p < 0.05$.

Results

Seventeen of the 46 ewes aborted within a few days of operation or died several weeks after operation from causes unrelated to treatment. Results for the remaining 29 ewes are presented in Fig. 2 and Table I. Fig. 2 shows the gestation for ewes in the four treatment groups. Two ewes aborted at 92 days of gestation. Ewes that carried only lambs with severed spinal cords had a significantly shorter gestation than ewes in the other treatment groups ($p < 0.05$). This effect was consistent except for one lamb that was delivered after a normal period. However, lambs with severed spinal cords that were twins of intact lambs were delivered after a normal gestation with their intact mates.

Two of the four lambs with pinched tracheas were very edematous and died at birth. Alcorn et al.[10] reported a similar phenomenon in lambs in which the trachea was ligated between 105 and 110 and 120 to 148 days of gestation. Two were born alive but were killed because of severe breathing difficulties. Lambs with severed spinal cords appeared quite normal grossly and had normal wool. The spinal cord of each lamb had been severed cervically at C1-3. Lungs did not float, verifying that these lambs did not breathe. All lambs had meconium in the intestines.

The weights of lambs in the different treatment groups are shown in Table I. Lambs with severed spinal cords weighed less than controls, presumably in association with the short gestation. However, weights of lambs with severed spinal cords that were twins to intact lambs did not differ significantly from those of their intact counterparts. Lambs with pinched tracheas had

greater weights than controls because of the excess fluid retained by these lambs.

Comment

The significant finding of this study is that transection of the spinal cord of fetal lambs resulted in a shortened gestation to about 128 days. However, in cases in which lambs with severed spinal cords were twins to an intact lamb, both were delivered after a normal period of gestation.

Our finding of shorter gestations in lambs with severed spinal cords was unexpected. Binns et al.[11] reported a congenital cyclopian type of malformation associated with prolonged gestation in fetuses of range sheep in Idaho that had ingested *Veratrum californicum* (skunk cabbage). The affected lambs had pituitary glands, although the neural connections were either missing or abnormal. Decapitated pig fetuses have a prolonged gestation of 124 to 125 days.[8] Decapitated sheep fetuses were born at term or later.[3] However, Honnebier and Swaab,[12] who examined the influence of anencephaly of human fetuses on length of gestation, reported that a number of these fetuses were born prematurely at 34 to 36 weeks of gestation.

In the present study, the lambs with severed cords were dead at or shortly after birth when found during frequent inspections. It seems unlikely that intrauterine death precipitated parturition in view of the fact that lambs with severed spinal cords and a normal twin were born after a normal gestation. Also lambs with severed spinal cords did not weigh less than the normal twin or other intact controls but did weigh more than the single lambs with severed spinal cords born at 128 days of gestation.

In studies by Liggins et al.[13] and Dawes et al.,[14] spinal cord transections were performed at 115 to 123 days of gestation. No effect on gestation was detected, since lambs were delivered either spontaneously at term or by hysterotomy.

In the present study, lambs with severed spinal cords and an intact twin had a normal gestation. A similar phenomenon was observed in pigs by Stryker and Dziuk,[8] who found that a litter consisting of a ratio of more than one intact fetus to four decapitated fetuses had a normal gestation. Length of gestation was normal when a hypophysectomized lamb was a twin to an intact lamb.[2] In all cases it appears that the intact fetus can override whatever effect decapitation or severing of the spinal cord has on length of gestation.

Several studies[15, 16] of litters of lambs that contain fetuses of different genetic background have found that the fetus of the genetically shorter gestation can often influence the length of gestation of its twin. Fetal endocrine changes in a mixed breed pregnancy were largely controlled by the fetal twin with a shorter gestation.[16]

The results of our study show that transection of the spinal cord of fetal lambs at the cervical level at 50 to 70 days of gestation causes a shortened length of gestation. This effect is overridden by the presence of an intact fetus. Because no endocrine assessments were made, we can only conjecture as to the cause of the shortened gestation. However, the results of this study do support the idea that neural input can affect the length of gestation.

REFERENCES

1. Liggins GC. Premature delivery of fetal lambs infused with glucocorticoids. J Endocrinol 1969;45:515-20.
2. Liggins GC, Kennedy PC, Holm LW. Failure of initiation of parturition after electrocoagulation of the pituitary of the fetal lamb. Am J Obstet Gynecol 1967;98:1080.
3. Lanman JT, Schaffer A. Gestational effects of fetal decapitation in sheep. Fertil Steril 1968;19:598-605.
4. Schriefer JA, Lewis PR, Miller JW. Role of fetal oxytocin in parturition in the rat. Biol Reprod 1982;27:362.
5. Dawood MY, Khan-Dawood FS, Ayromlooi J, Jobias M. Maternal and fetal plasma oxytocin levels during pregnancy and parturition in the sheep. Am J Obstet Gynecol 1983;147:584.
6. Alexander DP, Britton HG, Forsling ML, Nixon DA, Ratcliffe JG. Adrenocorticotrophin and vasopressin in the fetal sheep and the response to stress. In: Pierrepoint GP, ed. The endocrinology of pregnancy and parturition: experimental studies in the sheep. Cardiff, Wales: Alpha Omega Alpha, 1972:112-25.
7. Alexander DP, Forsling ML, Martin MJ, et al. The effect of maternal hypoxia on fetal hormone release in the sheep. Biol Neonate 1972;21:219-29.
8. Stryker JL, Dziuk PJ. Effects of fetal decapitation on fetal development, parturition and lactation in pigs. J Anim Sci 1975;40:282.
9. Dziuk PJ, Phillips TN, Graber JW. Halothane closed-circuit anesthesia in the pig. Am J Vet Res 1964;25:1773.
10. Alcorn D, Adamson TM, Lambert TF, Maloney JE, Ritchie BC, Robinson PM. Morphological effects of chronic tracheal ligation and drainage in the fetal lamb lung. J Anat 1977;123:649-60.
11. Binns W, James LF, Shupe JL, Everett G. A congenital cyclopian-type malformation in lambs induced by maternal ingestion of a range plant, veratrum californicum. Am J Vet Res 1963;24:1164-79.
12. Honnebier WJ, Swaab DF. The influence of anencephaly upon intrauterine growth of fetus and placenta and upon gestation length. J Obstet Gynaecol Br Commonw 1973; 80:577-88.
13. Liggins GC, Vilos GA, Campos GA, Kitterman JA, Lee CH. The effect of spinal cord transection on lung development in fetal sheep. J Dev Physiol 1981;3:267-74.
14. Dawes GS, Gardner WN, Johnston BM, Walker DW. Breathing in fetal lambs: the effect of brain stem section. J Physiol 1983;335:535-53.
15. Anderson GB, Bradford GE, Cupps PT. Length of gestation in ewes carrying lambs of two different breeds. Theriogenology 1981;16:119-29.
16. Kitts DD, Anderson GB, Bon Durant RH, Kindahl H, Stabenfeldt GH. Studies on the endocrinology of parturition: relative steroidogenesis in coexisting genetically dissimilar ovine fetuses, concomitant with the temporal patterns of maternal C_{18} and C_{19} steroids and prostaglandin F_2 release. Biol Reprod 1985;33:67-78.

The effects of proteolytic enzymes on in vitro ovulation in the rabbit

Yasunori Yoshimura, M.D., Rosemary Santulli, B.S., Susan J. Atlas, Ph.D., Shingo Fujii, M.D., and Edward E. Wallach, M.D.

Baltimore, Maryland

The involvement of proteolytic enzymes in follicle rupture was assessed by use of the in vitro perfused rabbit ovary. Streptokinase (10 and 100 units/ml) induced ovulation in the absence of gonadotropin. Ovulation failed to occur in contralateral control ovaries. The time of ovulation in streptokinase- and human chorionic gonadotropin—treated ovaries was similar, but significantly more ova from streptokinase-treated ovaries were immature ($p < 0.001$). Other ovaries were pretreated with trans-4-(aminomethyl)-cyclohexane-carboxylic acid, an inhibitor of the conversion of plasminogen to plasmin, and then perfused with human chorionic gonadotropin (50 IU). Ovulatory efficiency was significantly reduced by trans-4-(aminomethyl)-cyclohexane-carboxylic acid at 10 or 1 mmol/L ($p < 0.001$), but ovum maturity was unaffected. Aprotinin (100 or 10 μg/ml), a potent inhibitor of plasmin, significantly inhibited human chorionic gonadotropin–induced ovulation ($p < 0.001$) but did not affect oocyte maturation. Scanning electron microscopy of detergent-treated streptokinase-perfused ovaries revealed loosening and decomposition of collagen in the tunica albuginea. These results suggest proteolytic enzyme involvement in follicle rupture. (Am J Obstet Gynecol 1987;157:468-75.)

Key words: Perfused ovary, proteolytic enzymes, ovulation

Ovulation is a complex phenomenon, involving oocyte maturation, follicular steroidogenesis, rupture of the graafian follicle, and release of mature ova.[1] Follicle rupture is preceded by disruption of connective tissue in the apex of the follicle wall. The decomposition of this tissue has been postulated to be dependent on proteolytic enzyme activity.[2] Collagenolytic enzymes, present in rat graafian follicles in the process of ovulation, are thought to play a significant role in disruption of the follicle wall.[3] Strickland and Beers[4] have hypothesized that gonadotropins induce ovulation by stimulating the synthesis of a plasminogen activator in mature ovarian follicles. Plasminogen activator acts on plasminogen in the follicular fluid and tissue to produce plasmin, which, in turn, weakens follicular wall tissue. These observations have not, however, proved conclusively that follicle rupture is a direct result of the action of plasminogen activator and/or other proteolytic enzymes. In contrast, recent observations raise questions about the specific role of plasminogen activator in the process of ovulation.[5] Indomethacin can inhibit ovulation in the rat while failing to significantly reduce ovarian plasminogen activator activity. These data suggest that plasminogen activator may not be the critical proteolytic enzyme for follicle rupture. The role of proteolytic enzymes in the mechanism of ovulation thus remains to be clarified. An isolated perfused ovary preparation has been effective in studying local factors involved in the ovulatory process and in evaluating the effects of specific agents on phenomena associated with ovulation.[1] The present study was designed to determine the effects of proteolytic enzymes on the processes of follicle rupture and oocyte maturation by means of an in vitro perfused rabbit ovary model.

From the Department of Gynecology and Obstetrics, The Johns Hopkins University School of Medicine.

Supported by National Institutes of Health Grants HD-19430 and HD-05948 and by the Connelly and Mitchell and Lillian Duberstein Foundations.

Presented in part at the Thirty-second Annual Meeting of the Society for Gynecologic Investigation, Phoenix, Arizona, March 20-23, 1985.

Received for publication September 12, 1986; revised February 3, 1987; accepted February 17, 1987.

Reprint requests: Edward E. Wallach, M.D., Department of Gynecology and Obstetrics, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Material and methods

Fifty-two sexually mature New Zealand White female rabbits were isolated for a minimum of 3 weeks prior to the experimental procedure. The rabbits were caged individually under controlled light and temperature with free access to Purina Rabbit Chow and water. Rabbits weighing at least 3.5 kg were anesthetized with intravenous pentobarbital sodium (32 mg/kg), given heparin sulfate (120 units/kg) for anticoagulation, and then subjected to laparotomy. Overies were excluded from study if they appeared immature or if 50% or more of the surface follicles appeared hemorrhagic. Each ovarian artery was cannulated in situ, and the ovary with ovarian artery and vein and supportive adi-

Table I. Time of ovulation and oocyte characteristics: Ovaries perfused with or without streptokinase

	Streptokinase			
	100 units/ml	*10 units/ml*	*1 unit/ml*	*Control*
Ovaries perfused (n)	6	6	6	18
Ovaries ovulating (n)	6	6	0	0
Time of ovulation (hr) (mean ± SE)	7.19 ± 0.26	6.71 ± 0.48	—	—
Ovulated ova (n)	25	13	0	0
Germinal vesicle breakdown (%)	16.0	23.1	—	—
Degeneration (%)	32.0	23.1	—	—
Follicular oocytes (n)	13	17	20	53
Germinal vesicle breakdown (%)	15.4	23.5	20.0	13.2
Degeneration (%)	38.5	17.6	25.0	22.6

pose tissue was removed and placed in a perfusion chamber. Each ovary was perfused with 150 ml of tissue culture medium 199 (M. A. Bioproducts, Walkerville, Maryland) supplemented with insulin, heparin, streptomycin, and penicillin. The cannulation procedure and perfusion apparatus have been previously described in detail.[6]

Ovaries were periodically observed for evidence of follicle growth and rupture. A follicle was considered ruptured when the cumulus containing an ovum was observed protruding from the ovarian surface. The ovulated ovum surrounded by its cumulus mass was carefully recovered from the ovarian surface. At 12 hours after exposure to ovulation-inducing substances, follicular oocytes were recovered from mature follicles (>1.5 mm in diameter) and the experiment was concluded. Both ovulated ova and follicular oocytes were assessed for stage of maturity and signs of degeneration as previously described.[6] The degree of ovum maturity was expressed as the percentage of oocytes that had achieved germinal vesicle breakdown. Ovulatory efficiency, defined as the percentage of mature follicles that proceed to rupture, was calculated for each group.

Experiment 1: Effects of streptokinase on ovulation and oocyte maturation. The initial experiment was designed to determine if exposure to streptokinase, an exogenous plasminogen activator, would induce ovulation in the absence of gonadotropin. Streptokinase (Sigma Chemical Co., St. Louis, Missouri) at a concentration of 1, 10, or 100 units/ml was added to the perfusate of one ovary. The contralateral ovary of each rabbit was simultaneously perfused in a separate chamber with medium alone and served as a control. Six rabbits were used for each dose. Ovaries were observed throughout the 12 hours of perfusion for evidence of follicle growth and rupture. In a companion experiment, ovaries were perfused with human chorionic gonadotropin (hCG) (50 IU) or with streptokinase (100 units/ml) in order to compare the ovulatory efficiency in hCG- and streptokinase-induced ovulation.

Experiment 2: Effects of trans-4-(aminomethyl)-cyclohexane-carboxylic acid on hCG-induced ovulation and oocyte maturation. The second experiment was designed to determine if trans-4-(aminomethyl)-cyclohexane-carboxylic acid (tAMCHA), a potent inhibitor of the conversion of plasminogen to plasmin, blocks hCG-induced ovulation. tAMCHA (Sigma Chemical Co.) at a concentration of 1 or 10 mmol/L was added to the perfusate of one ovary. The contralateral ovary of each rabbit was simultaneously perfused in a separate chamber with medium alone and served as a control. Thirty minutes after the onset of perfusion, 50 IU of hCG was added to the perfusate of both ovaries. Six rabbits were used at each dose level. Perfused ovaries were observed for 12 hours after exposure to hCG.

Experiment 3: Effects of aprotinin on hCG-induced ovulation and oocyte maturation. The third experiment was designed to determine if aprotinin, a protease inhibitor, blocks hCG-induced ovulation. Aprotinin (Sigma Chemical Co.) at a concentration of 10 or 100 μg/ml was added to the perfusate of one ovary. The contralateral ovary, which served as a control, was perfused with medium alone. Thirty minutes after the onset of perfusion, 50 IU of hCG was added to the perfusate of both ovaries. Six rabbits were used at each dose level.

Experiment 4: Surface morphologic features of ovarian follicles perfused with streptokinase. After perfusion with 100 units/ml streptokinase, ovaries from four rabbits were treated with the detergent sodium dodecyl sulfate according to the method described by Martin and Miller-Walker.[7] The first ovulation in this system occurred at approximately 5 hours after the onset of perfusion. Therefore perfusion was terminated at 4 and 7 hours after exposure to streptokinase (100 units/ml) in order to observe the morphologic alterations of the follicle during the process of ovulation. Perfused ovaries were placed in 5% sodium dodecyl sulfate in distilled water for 5 to 6 minutes and fixed

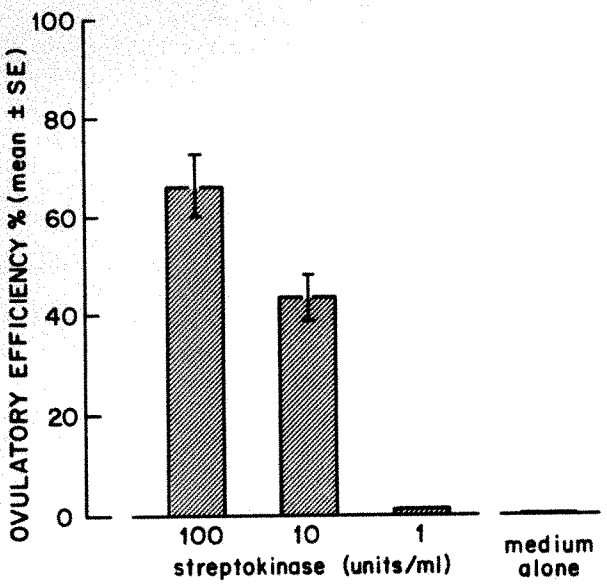


Fig. 1. Ovulatory efficiency of ovaries perfused with or without streptokinase. Ovaries that served as controls were perfused with medium alone. The contralateral experimental ovaries were perfused with streptokinase at a concentration of 1, 10, or 100 units/ml.

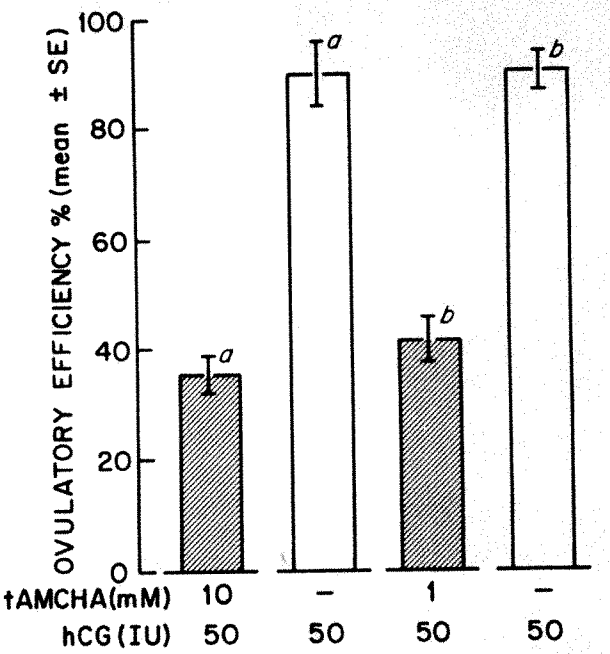


Fig. 2. Ovulatory efficiency of ovaries perfused with hCG plus tAMCHA or with hCG alone. Ovaries that served as controls were perfused with medium alone. The contralateral experimental ovaries were perfused with tAMCHA at a concentration of 1 or 10 mmol/L. Thirty minutes after the onset of perfusion hCG (50 IU) was added to the perfusate of both ovaries. Groups identified by like letters were significantly different (*a*, *b* = $p < 0.001$).

Table II. Time of ovulation and oocyte characteristics: Ovaries perfused with hCG or with streptokinase

	hCG (50 IU)	*Streptokinase (100 units/ml)*
Ovaries perfused (n)	6	6
Ovaries ovulating (n)	6	6
Time of ovulation (hr) (mean ± SE)	7.22 ± 0.28	6.95 ± 0.37
Ovulatory efficiency (mean ± SE)	82.3 ± 5.2*	60.5 ± 6.6*
Ovulated ova (n)	43	27
Germinal vesicle breakdown (%)	93.0†	18.5†
Degeneration (%)	14.0	22.2
Follicular oocytes (n)	10	21
Germinal vesicle breakdown (%)	90.0†	14.2†
Degeneration (%)	22.2	23.8

*Significantly different ($p < 0.02$).
†Significantly different ($p < 0.001$).

in 2.5% glutaraldehyde in 0.1 mol/L cacodylate buffer (pH 7.4) for 3 hours. The fixed ovaries were treated with 0.02% deoxyribonuclease for 1 hour at 20° C and then processed for examination with an Amray 1200 scanning electron microscope.

Statistical analysis. All data regarding the time of ovulation and ovulatory efficiency are presented as the mean ± SE. Statistical analysis was determined by use of Student's *t* test. The χ^2 test with Yates' correction was used to assess the percent degeneration and the percent germinal vesicle breakdown of follicular oocytes and ovulated ova.

Results

Experiment 1: Effects of streptokinase on ovulation and oocyte maturation. Streptokinase at a concentration of 10 or 100 units/ml, but not at 1 unit/ml, induced ovulation in the absence of hCG, whereas ovulation did not occur in any of the control ovaries (Table I and Fig. 1). The majority of ova ovulated in vitro did not progress beyond the germinal vesicle intact stage after treatment with streptokinase. In contrast, > 80% of ovulated ova from hCG-treated ovaries achieved an advanced stage of maturity (Table II). The ovulatory efficiency was significantly increased when ovaries were perfused with concentrations of streptokinase above 10 units/ml ($p < 0.05$). The time interval from ovulatory stimulus to ovulation was similar in streptokinase- and hCG-treated ovaries (Table II). At 100 units/ml of streptokinase, ovulatory efficiency was, however, significantly lower than that observed in the contralateral hCG-treated ovaries ($p < 0.02$). Examination of ovulated ova and follicular oocytes for evidence of degeneration showed no significant difference between

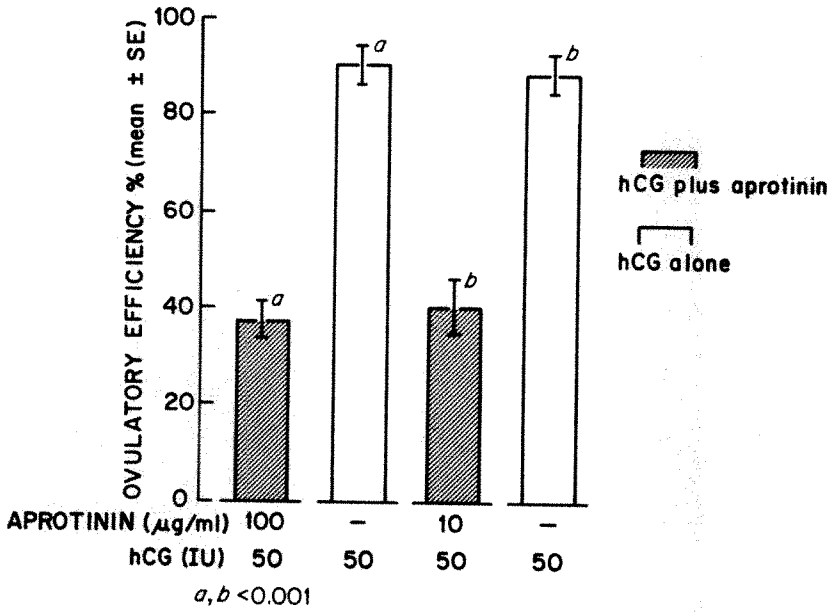


Fig. 3. Ovulatory efficiency of ovaries perfused with hCG plus aprotinin or with hCG alone. One ovary of each rabbit was perfused with medium alone. The contralateral, experimental ovary was perfused with aprotinin at a concentration of 10 or 100 μg/ml. Thirty minutes after the onset of perfusion, hCG (50 IU) was added to the perfusate of both ovaries. Groups identified by like letters were significantly different (*a*, *b* = $p < 0.001$).

Table III. Time of ovulation and oocyte characteristics: Ovaries perfused with hCG plus tAMCHA or with hCG alone

	tAMCHA (10 mmol/L)	*tAMCHA (1 mmol/L)*	*hCG (50 IU)*
Ovaries perfused (n)	6	6	12
Ovaries ovulating (n)	6	6	12
Time of ovulation (hr) (mean ± SE)	8.67 ± 0.24	9.22 ± 0.32*	7.81 ± 0.24*
Ovulated ova (n)	12	14	69
Germinal vesicle breakdown (%)	100	92.9	92.8
Degeneration (%)	25.0	28.6	17.4
Follicular oocytes	22	19	7
Germinal vesicle breakdown (%)	81.8	63.2	85.7
Degeneration (%)	40.9	31.6	28.6

*Significantly different ($p < 0.01$).

groups within each series of experiments (Tables I and II).

Experiment 2: Effects of tAMCHA on hCG-induced ovulation and oocyte maturation. Addition of tAMCHA at a concentration of 1 or 10 mmol/L to the perfusate, together with 50 IU of hCG, resulted in an effective blockade of ovulation when comparison was made with ovaries exposed to hCG alone. Ovulatory efficiency was significantly reduced in ovaries exposed to tAMCHA as compared with control ovaries ($p < 0.001$) (Fig. 2). Addition of tAMCHA at 1 mmol/L to the perfusate significantly delayed the time interval to ovulation in ovaries exposed to hCG when compared with control ovaries treated with hCG alone ($p < 0.01$) (Table III). The percentage of ovulated ova and follicular oocytes achieving germinal vesicle breakdown did not differ significantly between tAMCHA-treated and control ovaries. Although the percentage of ovulated ova and follicular oocytes that showed evidence of degeneration was increased in tAMCHA-treated ovaries as compared with control ovaries, these differences were not significant.

Experiment 3: Effects of aprotinin on hCG-induced ovulation and oocyte maturation. Aprotinin at a concentration of 10 or 100 μg/ml significantly inhibited hCG-induced ovulation ($p < 0.001$) (Fig. 3). The mean

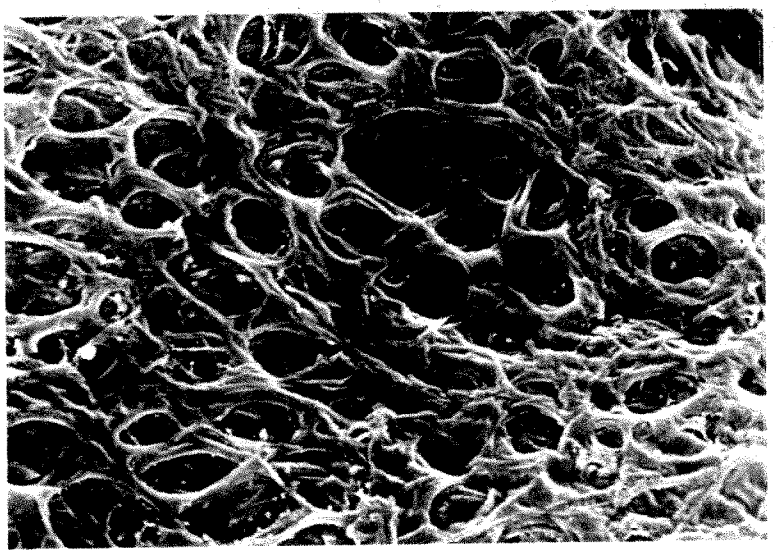


Fig. 4. Surface morphologic features of ovarian follicles at 4 hours after exposure to streptokinase. The honeycomb-like structure characteristic of the basal lamina is evident. (Original magnification ×2000.)

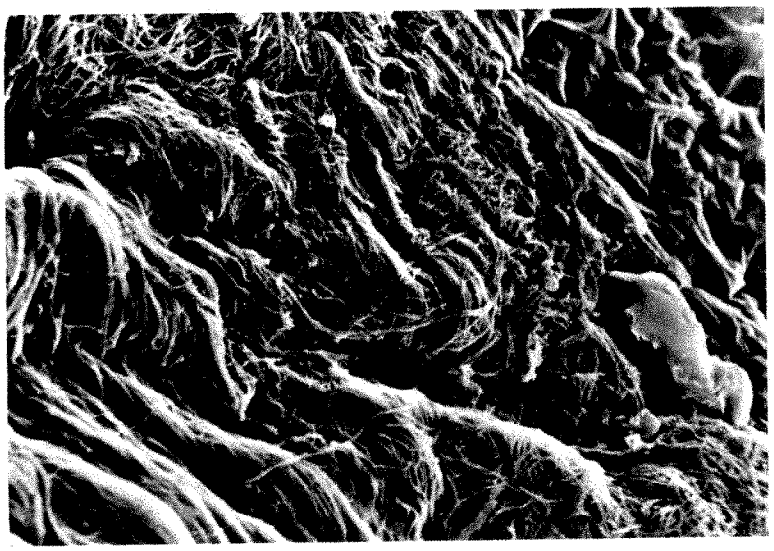


Fig. 5. Surface morphologic features of ovarian follicles at 7 hours after exposure to streptokinase. Degenerative changes in the basal lamina have exposed collagen fibrils. (Original magnification ×2000.)

time of ovulation was delayed in aprotinin-treated ovaries as compared with control ovaries. This difference, however, was not significant (Table IV). Aprotinin at both concentrations did not affect the maturity of ovulated ova and follicular oocytes in ovaries perfused with hCG. The percentage of ovulated ova and follicular oocytes that showed evidence of degeneration was greater in aprotinin-treated ovaries than in control ovaries; however, this difference was not significant.

Experiment 4: Surface morphologic features of ovarian follicles perfused with streptokinase. The detergent sodium dodecyl sulfate is effective in removing surface epithelial cells in the rabbit ovary, allowing examination of the integrity and distribution of subepithelial tissue in the preovulatory follicle. At 4 hours after exposure to streptokinase, sodium dodecyl sulfate treatment revealed the honeycomb-like architecture characteristic of the basal lamina (Fig. 4). Degenerative change in the subepithelial connective tissue was not prominent at the apex of the follicle. At 7 hours after streptokinase exposure, sodium dodecyl sulfate treatment revealed defects associated with degeneration of the basal lamina. Collagen fibrils located near the lateral margin of the apex were compact, forming several bun-

Table IV. Time of ovulation and oocyte characteristics: Ovaries perfused with hCG plus aprotinin or with hCG alone

	Aprotinin (100 μg/ml)	*Aprotinin (10 μg/ml)*	*hCG (50 IU)*
Ovaries perfused (n)	6	6	12
Ovaries ovulating (n)	6	6	12
Time of ovulation (hr) (mean ± SE)	7.92 ± 0.59	8.76 ± 0.49	7.83 ± 0.24
Ovulated ova (n)	13	14	66
Germinal vesicle breakdown (%)	92.3	92.9	92.4
Degeneration (%)	23.1	28.6	15.2
Follicular oocytes (n)	21	19	7
Germinal vesicle breakdown (%)	95.2	78.9	85.7
Degeneration (%)	23.8	26.3	28.6

dles or wide bands. The apical region was covered by distended thin collagen fibrils, resulting from degeneration or decomposition of connective tissues in the tunica albuginea (Fig. 5).

Comment

Many serine proteases and protease inhibitors that are thought to be involved in the process of follicle rupture may be present physiologically in vivo.[8] Plasma serine protease inhibitors such as α_1-antiplasmin, α_2-macroglobulin, and α_1-antitrypsin react rapidly and irreversibly with plasmin, inhibiting its protease activity.[9] Such humoral factors are excluded in this study because the perfusion system used lacks plasma or cellular blood components. This ovarian perfusion preparation can serve as an effective model for exploring the direct effects of agents on the process of ovulation while eliminating the possibility of systemic influences.[1, 10]

The sequence of events following gonadotropin stimulation that culminates in rupture of the graafian follicle and release of a mature ovum has been the subject of considerable attention. Steroid hormones, prostaglandins, inflammatory mediators including proteolytic enzymes, and ovarian smooth muscle have been implicated as participants in the process of follicle rupture.[1] The cumulative data indicate that an essential prerequisite for follicle rupture is the decomposition of the collagenous layers of the follicle.[2, 3, 7]

The present study of the in vitro perfused rabbit ovary preparation has demonstrated that streptokinase, an exogenous plasminogen activator, induces ovulation in the absence of gonadotropin. Strickland and Beers[4] have proposed the hypothesis that gonadotropins induce ovulation by stimulating the synthesis of a plasminogen activator in mature ovarian follicles. The data reported herein support the involvement of plasminogen activator in the mechanism of follicle rupture. The failure of streptokinase to induce ovulation with an efficiency comparable to that of hCG suggests that other components within the preovulatory follicular environment may also be required for follicle rupture. The relative inefficiency of streptokinase when compared with hCG may be related to the bacterial origin of this enzyme; native mammalian tissue plasminogen activator produced in response to hCG may be more active in vivo.

Streptokinase exerts its plasminogen activator activity after formation of a complex with plasminogen.[11] That the addition of streptokinase alone to the perfused ovary can induce ovulation implies that the ovary contains plasminogen, which binds to the exogenous streptokinase and produces an activated complex responsible for the observed effects. Streptokinase treatment was associated with degradation and decomposition of collagen fibrils within the tunica albuginea of preovulatory follicles. The results of the present study suggest that the plasminogen activator–plasmin system may lead to the activation of collagenase, thereby resulting in the disruption of follicular integrity necessary for ovum release.

The latent period between exposure to the stimulatory agent and time of ovulation did not differ significantly between ovaries treated with hCG and streptokinase; nonetheless significant differences in oocyte maturational stage were observed. These results imply that the appropriate factors necessary for oocyte maturation are lacking in streptokinase-induced ovulation. The failure of ova to undergo germinal vesicle breakdown and further maturation after exposure to streptokinase indicates that the phenomena of oocyte maturation and follicle rupture are dissociated in ovaries treated with plasminogen activator. Other agents have also been shown to evoke follicle rupture without leading to ovum maturation in this system.[10] Furthermore, this study demonstrates that exposure to serine protease inhibitors significantly reduces ovulatory efficiency in ovaries treated with hCG but does not affect oocyte maturation elicited by hCG administration.

tAMCHA has been reported to inhibit the activation

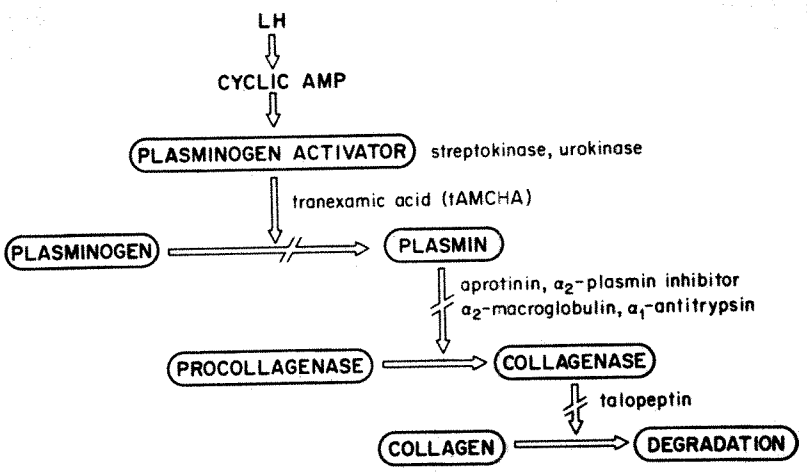


Fig. 6. Proposed scheme for involvement of proteolytic enzymes in disruption of the wall of the ovarian follicle.

of plasminogen to plasmin and fibrinolysis by active plasmin.[12] Aprotinin, a pancreatic trypsin inhibitor, inhibits plasmin and other serine proteases.[13] Ovulatory efficiency after hCG administration is significantly reduced by exposure to serine protease inhibitors. These data are consistent with the report that various microbial protease inhibitors completely block ovulation in the explanted hamster ovary.[14] The results of the present study provide further support for the role of serine protease(s) in the process of follicle rupture. However, the failure of antiplasmin agents to completely block hCG-induced ovulation suggests that other components, in addition to the plasmin-generating system, participate in follicle rupture.

Espey[8] has hypothesized that the preovulatory gonadotropin surge may induce mammalian ovulation by initiating an inflammation-like reaction in mature ovarian follicles. The role of common mediators of inflammation, such as prostaglandins and histamine, in the process of follicle rupture has been demonstrated in previous studies with the perfused rabbit ovary preparation.[10] Other reports provide evidence that ovulation can occur despite significant inhibition of plasminogen activator activity.[5] The plasminogen activator–plasmin system may contribute to collagenolysis in connective tissues of the ovarian follicles, but the present data cannot exclude the possibility of the involvement of inflammatory mediators other than the plasmin-generating system in the process of follicle wall disruption.

In conclusion, we propose a working model for the mechanism of follicular wall disruption in mammals (Fig. 6), which is a modification of the model suggested by Espey.[8] After the gonadotropin surge, plasminogen activator, which is responsible for plasmin formation, is produced by granulosa and theca cells.[15, 16] An elevation of the level of plasminogen activator in the follicle yields increasing intrafollicular concentrations of plasmin. The presence of plasmin and other serine proteases leads to the activation of latent collagenase, resulting in the degradation of collagenous connective tissue in the follicle. A series of enzyme systems acting locally alters the structural integrity of the follicle wall and facilitates follicle rupture.

REFERENCES

1. Yoshimura Y, Wallach EE. Studies of the mechanism(s) of mammalian ovulation. Fertil Steril 1987;47:22-34.
2. Espey LL. Ovarian proteolytic enzymes and ovulation. Biol Reprod 1974;10:216-35.
3. Morales TI, Woessner JF Jr, Marsh JM, LeMaire WJ. Collagen, collagenase and collagenolytic activity in rat Graafian follicles during follicular growth and ovulation. Biochim Biophys Acta 1983;756:119-22.
4. Strickland S, Beers WH. Studies on the role of plasminogen activator in ovulation. J Biol Chem 1976;251:5694-702.
5. Espey L, Shimada H, Okamura H, Mori T. Effect of various agents on ovarian plasminogen activator activity during ovulation in pregnant mare's serum gonadotropin-primed immature rats. Biol Reprod 1985;32:1087-94.
6. Kobayashi Y, Wright KH, Santulli R, Wallach EE. Ovulation and ovum maturation in the rabbit ovary perfused in vitro. Biol Reprod 1981;24:483-90.
7. Martin GG, Miller-Walker C. Visualization of the three-dimensional distribution of collagen fibrils over preovulatory follicles in the hamster. J Exp Zool 1983;225:311-9.
8. Espey LL. Ovulation as an inflammatory reaction—a hypothesis. Biol Reprod 1980;22:73-106.
9. Mullertz S, Clemmensen I. The primary inhibitor of plasmin in human plasma. Biochem J 1976;159:545-53.
10. Kitai H, Kobayashi Y, Santulli R, Wright KH, Wallach EE. The relationship between prostaglandins and histamine in the ovulatory process as determined with the in vitro perfused rabbit ovary. Fertil Steril 1985;43:646-51.
11. Castellino FJ, Violand BN. The fibrinolytic system—basic considerations. Prog Cardiovasc Dis 1979;21:241-54.
12. Iwamoto M. Plasminogen-plasmin system. IX. Specific

binding of tranexamic acid to plasmin. Thromb Diath Haemorrh 1975;33:573-85.
13. Haberland G, McConn R. A rationale for the therapeutic action of aprotinin. Fed Proc 1979;38:2760-7.
14. Ichikawa S, Morioka H, Ohta M, Oda K, Murao S. Effect of various proteinase inhibitors on ovulation of explanted hamster ovaries. J Reprod Fertil 1983;68:407-12.
15. Wang C, Leung A. Gonadotropins regulate plasminogen activator production by rat granulosa cells. Endocrinology 1983;112:1201-7.
16. Canipari R, Strickland S. Plasminogen activator in the rat ovary. Production and gonadotropin regulation of the enzyme in granulosa and theca cells. J Biol Chem 1985;260:5121-5.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1987 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 156 (January-June) and Vol. 157 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Disruption of embryonic and fetal development due to preimplantation chemical insults: A critical review

Philip M. Iannaccone, M.D., Ph.D., Nancy L. Bossert, B.A., and Camille S. Connelly, B.A.
Chicago, Illinois

Descriptive teratology has developed several fundamental precepts, two of which can now be challenged on the basis of experimental evidence. The first is that prior to implantation the developing embryo is not susceptible to survivable defects from chemical injury. The second is that developmental defects cannot be due to mutational events since rare events seem unlikely to explain alterations in large populations of cells. This review presents current experimental evidence demonstrating that the effects of chemical exposure on blastocyst stage embryos may be manifest long after the time of insult and that subtle nonlethal mutations may have a role in poor fetal performance after early chemical exposures. (Am J Obstet Gynecol 1987;157:476-84.)

Key words: Preimplantation, mammalian embryology, teratology, pregnancy outcome

The tragic consequences of thalidomide use during pregnancy made the susceptibility of the developing fetus to chemical exposure abundantly clear. Many chemicals, some found frequently in our environment, have been described as teratogens. Many of these teratogens are now avoided and thus represent less of a threat to modern pregnancies than at other times. However, maternal smoking remains a major source of fetal exposure to environmental toxicants during gestation. Simpson[1] reported the first clear association of maternal smoking with adverse effects on the developing fetus. This work demonstrated a significant dose-dependent association between maternal smoking and premature birth. A clear association between maternal smoking and perinatal mortality, spontaneous abortion, and sudden infant death syndrome as well as maternal complications has been demonstrated.[2] Traditional studies of this association have examined mechanisms of fetal disruption during organogenesis and later gestational periods. Many studies aimed at understanding the relationship of smoking to pregnancy complications, both maternal and fetal, have concentrated on the direct toxic effects of nicotine in late gestation.[3, 4] However, tobacco and tobacco smoke contain a number of highly mutagenic substances. Indeed, nicotine itself can be metabolized to form mutagens.[5]

It has been generally held that prior to implantation, the embryo is insensitive to developmental program shifts as a result of chemical exposure.[6, 7] Recent studies have shown this concept to be an oversimplification. In this review we describe the possible role that mutations during preimplantation development may play in poor pregnancy outcome.

Preimplantation development

The preimplantation period of development in mammals is the time from fertilization to implantation (Fig. 1). The length of this period varies with different species. The preimplantation period is about 7 days in length in humans, 4 days in mice, and 5 days in rats. In many mammalian species, at this stage of development, the embryo is 100 μm in diameter, is associated with two polar bodies remaining from meiotic division, and is surrounded by a chemically complex structure called the zona pellucida. The zona pellucida is a highly refractile, noncellular coat consisting of mucopolysaccharides and sialic acid residues. The zona pellucida is important to early development for several reasons. Penetration of the egg by the fertilizing spermatozoon is accompanied by changes in the zona that help prevent polyspermic fertilization. The zona provides a constraint to cleavage and ensures that as the blastomeres divide they remain together and in the proper orien-

From the Department of Pathology and Northwestern University Cancer Center, Northwestern University Medical School.
This work was performed in part with support from United States Public Health Service Grants CA29078 and ES03498 as well as from March of Dimes Birth Defects Foundation Grant 15-49.
Reprint requests: Dr. P. M. Iannaccone, Department of Pathology, Northwestern University Medical School, 303 East Chicago Ave., Chicago, IL 60611.

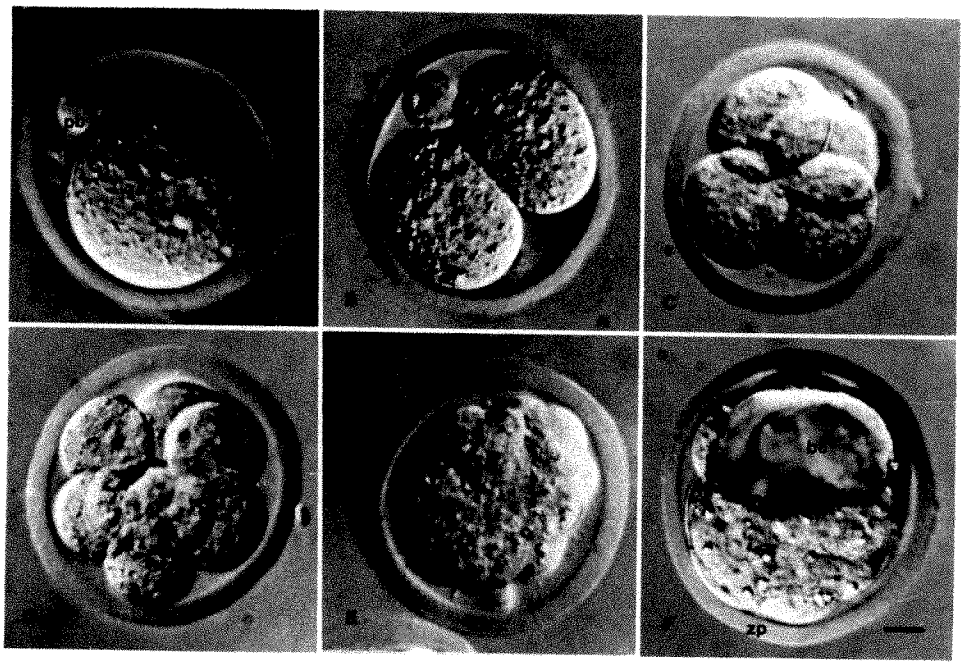


Fig. 1. Photomicrographs of living preimplantation mouse embryos (Hoffman modulation contrast optics). *A*, Pronuclear stage, single-cell embryo approximately 9 hours after fertilization. Both the maternal *(m)* and paternal *(p)* pronuclei are visible in the center of the embryo. The embryo is surrounded by the zona pellucida (*zp* in panel *F*). One polar body *(pb)* is still visible. *B*, First cleavage stage, two-cell embryo approximately 33 hours after fertilization. *C*, Four-cell embryo, approximately 48 hours after fertilization. *D*, Eight-cell embryo, before compaction, approximately 54 hours after fertilization. *E*, Postcompaction, eight-cell embryo approximately 60 hours after fertilization. *F*, Blastocyst stage embryo, approximately 86 hours after fertilization. All embryos are approximately 100 μm in diameter. *Bar* = 20 μm.

tation. The zona also prevents the embryo from adhering to the wall of the oviduct.

Mitotic cleavage division occurs entirely in the oviduct of most mammals, including man. The dividing embryo traverses the oviduct as the combined result of ciliary motion and muscular contraction. Divisions are symmetrical and reductive, resulting in a geometric increase in the number of blastomeres while the embryo remains a constant size. Prior to the eight-cell stage embryos in some mammalian species appear to have equivalent, totipotent blastomeres.[8, 9]

During early cleavage (the late eight-cell stage in the mouse) the embryo undergoes compaction, the morphogenetic process by which an embryo progresses from a collection of rounded, loosely associated blastomeres into a spherical embryo.[10] Individual blastomeres change shape and flatten against one another, thus maximizing the amount of cell-to-cell contact. Compaction appears to involve three steps: cell surface recognition and attachment of a ring of lateral microvilli to adjacent blastomeres, subsequent microfilament shortening in these lateral microvilli, and maintenance of the compacted, polarized state, which results in apical localization of microvilli on each blastomere. Compaction seems to be reversible and can be reinduced, but polarization of microvilli can occur only once.[10]

Prior to compaction, sister blastomeres of mouse embryos exhibit ionic coupling and possess cytoplasmic bridges that admit both fluorescein and horseradish peroxidase, a large molecule unable to cross gap junctions. Blastomeres from different mitotic pairs do not have demonstrable intercellular pathways. Intercellular communication mediated by gap junctions is first detected in the compacted eight-cell mouse embryo. At this stage, ionic coupling is observed and fluorescein injected into one cell is distributed to all eight cells of the embryo.[11] Gap junction formation does not appear to require concurrent protein synthesis since inhibition of protein synthesis by cycloheximide treatment fails to block gap junction construction.[12]

The increase in cell number from the eight-cell to the 16-cell stage necessitates that a certain number of cells occupy the interior of the morula and are completely separated from the outside by other cells. The external cells create a microenvironment in which the internal cells develop. These microenvironmental differences due to geographic location are the basis for the "inside-outside" hypothesis formulated by Tar-

kowski and Wroblewska.[8] This hypothesis states that the differentiation of blastomeres into trophoblastic and inner mass cells depends on the position of the blastomeres within the embryo. The "inside-outside" hypothesis is best supported by the experiments of Hillman et al.[13] In these experiments, embryos and parts of embryos were combined during early preimplantation stages. Each blastomere of a four-cell embryo could form both the trophectoderm and the inner cell mass of the blastocyst. However, when individual blastomeres of a four-cell embryo were placed on the outside of other four-cell embryos, they usually formed the outside layer of the blastocyst and went on to develop into the trophoblast and the yolk sac. Similar results were seen with eight-cell embryos.

Subsequent to compaction, polarization, and gap junction formation, zonular tight junctions are formed between peripheral blastomeres. The resulting barrier allows for the development of a fluid-filled cavity (blastocele) within the embryo. This is the beginning of the blastocyst stage of development. The blastocele enlarges as cells in this area degenerate, until the embryo resembles a hollow sphere. It comprises a single layer of large, flat cells on the periphery, called the trophectoderm or trophoblast, and an eccentric aggregate, known as the inner cell mass. The trophectoderm will develop into extraembryonic tissues, including the placenta, and is responsible for implantation and invasion of the maternal uterine tissue. The inner cell mass will develop into the fetus.

Implantation begins in the late blastocyst stage of the developing embryo. Implantation can be viewed as a process with at least three major phases. The first is the attachment phase, in which receptors on the trophectoderm are responsible for tight binding to endometrial epithelium. The second phase is invasion. In man, the trophectodermal cells invade through the basement membrane of the uterine epithelium to establish an implantation site in the stroma of the endometrium. In the mouse and rat, the endometrium engulfs the attached blastocyst, which results in an interstitial implantation. The third phase is the endometrial response to the implanted embryo. In a few eutherian species (including man, primates, and murine rodents) the uterine stromal cells undergo a specific reaction called decidualization, whereby the stromal cells in the immediate area of the embryo become large, eosinophilic, and transcriptionally active. The cells of the decidual swelling may be important in support of pregnancy and in suppression of immune rejection of the implanted embryo.[14, 15] Furthermore, decidual prolactin may be involved in the regulation of amniotic fluid volume,[16] and decidual luteotropin (a prolactin-like hormone in the rat) has been implicated in the maintenance of the corpus luteum.[17, 18]

Embryonic dysfunction

There is a significant waste of human life due to fetal loss. While there are approximately 3×10^6 live births a year in the United States, there are approximately 6×10^5 spontaneous abortions. Ten percent of the live births are premature and 13% of the infants have low birth weights. There are approximately 3×10^4 perinatal deaths, and about 2.6×10^4 children do not survive into their second year.[19]

The causes of poor pregnancy outcome have long been sought. One potential source of disruption of pregnancy is the exposure of pregnant women to toxic substances during the pregnancy. Of particular concern is the exposure of women in the very early periods of pregnancy. There has been a scarcity of animal studies suited to the study of toxic exposure in early pregnancy, to some extent because of the traditional view that preimplantation stages of development are refractory to the consequences of toxic insult. The general presumption that early stages are either killed or left unaffected to implant and develop normally has been shown to be an oversimplification. The blastocyst stage of development has, for example, been shown to be sensitive to cyclophosphamide,[20] heavy metals, and trypan blue.[21] Early embryos seem to exhibit differential sensitivity to treatment, dependent on specific developmental stage. For example, mouse embryos at the early blastocyst stage (day 3) are much more sensitive to the lethal effects of actinomycin D than at any of the other preimplantation stages. Actinomycin D prevents cleavage at these other preimplantation stages but does not cause cell death.[22] This differential sensitivity cannot be attributed to the rate or extent of drug accumulation since the less sensitive late blastocysts take up actinomycin D more rapidly and accumulate it more than do the more sensitive early blastocysts. Blastocysts are also more susceptible to the acute effects of methylmercury than morulae. Methylmercury exerts acute effects at high concentrations by arresting preimplantation development or by collapsing blastocysts, and at low concentrations it interferes with development of the inner cell mass.[23] Furthermore, methylnitrosourea will not prevent progression of morula stage embryos to the blastocyst stage at concentrations that cause blastocyst dysfunction.[24]

Within individual blastocysts, inner cell mass cells and trophectoderm may have different sensitivities to chemical insult. Adams et al.[25] administered various substances parenterally, including diethylstilbestrol, antimitotic agents, polyfunctional alkylating agents, and purine and pyrimidine analogues, to experimental animals during the preimplantation period of pregnancy and found that inner cell mass cells on the whole were more noticeably affected. This distinctive susceptibility of the inner cell mass and trophectoderm is presumably

the expression of differences in both type and rate of metabolic processes, whereby the more active and rapidly proliferating tissue of the embryo proper suffers adverse effects more readily. Alternatively, the difference in susceptibility between the two cell populations may reflect a difference in the recuperative potential (such as compensatory growth) of the two tissues.

The deleterious effects of preimplantation treatment range from increased intrauterine mortality to fetal growth retardation to actual malformations. For example, exposure to inhaled chloroform from days 1 to 7 significantly impaired the ability of female mice to maintain pregnancy but was not significantly teratogenic.[26] Mean fetal body weight and crown-rump length were decreased significantly among the surviving offspring. Intramuscular injection of nickel subsulfide to pregnant rats on day 6 of gestation also caused a significant increase in intrauterine mortality, although no skeletal or visceral anomalies were observed in pups in the treated or control litters.[27] Single intraperitoneal injections of nickel chloride to pregnant mice on day 1, 2, 3, 4, 5, or 6 of gestation resulted in decreased litter size, diminished fetal body weights, a larger frequency of both early and late resorptions, and an increased frequency of stillborn and abnormal fetuses, when compared with results in control groups.[28]

Takeuchi[29] found that single intraperitoneal injections of methylnitrosourea to mice on day 0, 1, 2, 3, or 4 of gestation resulted in no significant differences in the number of implantation sites between all the methylnitrosourea-treated groups and the control groups. However, the mean fetal death rates were significantly increased in methylnitrosourea-treated groups compared with the control groups, with early fetal deaths contributing substantially to these higher rates. The mean malformation rates were also significantly increased in groups treated with 10 mg/kg of methylnitrosourea on day 2, 3, or 4 as compared with the control groups. Exencephaly, cleft palate, and abnormal tail were prevalent malformations in each group. Cyproterone acetate treatment of pregnant mice[30] on day 2 resulted in a significant increase in rate of intrauterine mortality, exencephaly, and heart abnormalities, a dose-dependent increase in cleft palate and urinary tract abnormalities, and a concentration-dependent decrease in fetal weight. Medroxyprogesterone treatment on day 2 was followed by sporadic increases in dead and resorbed fetuses, a decrease in fetal weight, and an increase in the rates of cleft palate and malformed or abnormally developed fetuses.[30] Storeng and Jonsen[31] found a single intraperitoneal injection of ricin to mice on day 1, 2, or 3 of pregnancy had no significant effect on the implantation frequency and the number of live fetuses obtained from treated mothers. However, the number of abnormal fetuses in the ricin-treated groups exceeded that of the control groups, with exencephaly as one of the abnormalities observed. Exencephaly is believed to be induced by some toxic agents during the period of organogenesis, but Storeng and Jonsen hypothesize that the cases in this study may be due to an early effect of ricin on the preimplantation embryos with subsequent expression in later stages of fetal development.

Various mechanisms might be the cause of the deleterious effects of preimplantation treatment. Some chemicals may damage certain maternal tissues that are necessary for the maintenance of normal embryonic development; some chemicals may cause reduced maternal food intake; but in either case growth-retarded or malformed fetuses may result from damaged maternal functions. To test whether cyclophosphamide interferes predominantly with the development of the preimplantation embryo or with the decidualization of the uterus of the mother, rat embryos from treated mothers were transferred to untreated recipients and embryos from untreated mothers were transferred to pretreated foster mothers.[32] The results showed that when treated blastocysts were transferred to untreated mothers the overall implantation rate was not significantly different from that in the untreated, transferred control group. However, the percentage of live fetuses was significantly decreased and the resorption rate was significantly increased when compared with those of the control group. When untreated blastocysts were transferred to treated foster mothers, the implantation rate and the percentage of live fetuses were significantly decreased and the resorption rate was significantly increased when compared with that of the control group. The percentage of live fetuses was reduced to the same extent in both treated groups.

Inner cell mass cell sensitivity

The effect of chemical exposure in the preimplantation period does not appear to be simply a matter of inner cell mass cell death, particularly at sublethal concentrations of the offending agent. Snow and Tam[33] showed that the mouse embryo could be reduced to approximately 10% of its normal size after chemical exposure at a time when it was about to begin organogenesis, but it was nearly normal in size again before that phase of development was complete. Furthermore, much of the recovery in size was accomplished within 48 hours of the infliction of the damage. During this period of compensatory growth the embryo may be susceptible to irreversible morphogenetic damage. Moreover, the inner cell mass may contain as few as three or four cells that are fated to develop into the fetus.[34] Thus any significant cell death within this population should result in the complete disorganization of the embryo.

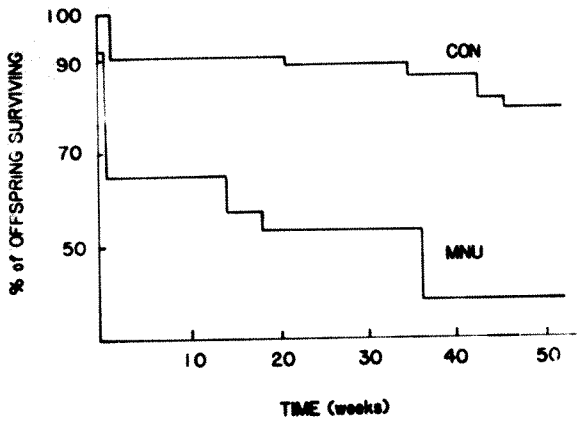


Fig. 2. Survival curves of offspring derived from embryos exposed to N-methyl-N-nitrosourea *(MNU)* or to the solvent dimethyl sulfoxide *(CON)*. The time after birth is shown in weeks. (n = 22 for *MNU;* n = 58 for *CON*). (Reprinted with permission of Cancer Research, Inc. From Iannaccone PM. Long-term affects of exposure to methylnitrosourea on blastocysts following transfer to surrogate female mice. Cancer Res 1984;44:2785-9.)

Combined in vitro, in vivo studies

Direct acting mutagens. Obviously, it is important to establish whether chemical exposures such as those discussed above have direct embryonic or fetal effects as opposed to maternal effects with subsequent poor pregnancy outcome. We have examined the issue of preimplantation sensitivity to chemical exposure with a combined in vitro, in vivo approach. In these experiments, embryos were removed from the uteri of pregnant mice at the blastocyst stage of development and exposed under precisely defined conditions in a dish. The embryos were then removed to the reproductive tract of pseudopregnant surrogate mothers. These experiments have included exposures to N-methyl-N-nitrosourea (methylnitrosourea) and the metabolites of benzo[a] pyrene. Methylnitrosourea is a direct-acting mutagen (that is, metabolism to an activated form is not required for mutagenesis) that alkylates large molecules including deoxyribonucleic acid. The manner in which this compound causes mutations is chemically analogous to the manner in which nicotine metabolites, the so-called tobacco-specific nitrosamines, cause mutations.[35, 36] Exposure to this chemical at concentrations well below lethal levels results in a dose-dependent decrease in the frequency of normal implantation without an effect on the total number of implantations (both normal and abnormal).[37] Blastocyst exposure to methylnitrosourea results in dose-dependent decreases in midgestational body length and an increase in the frequency of gross dysmorphogenesis at day 12 and day 15.[38] Exposure of gravida on day 3 (blastocyst stage in the mouse) results in malformations of fetuses in a dose-dependent manner assessed at day 18.[29] The malformations at each of the gestational points studied were related to growth retardation. When the exposed blastocysts were implanted in the uteri of surrogates that were then allowed to deliver their offspring, there was a profound dose-dependent decrease in the live birth rate. The offspring derived from the blastocysts that were exposed to methylnitrosourea had a threefold higher mortality rate than the offspring derived from solvent-exposed control blastocysts (Fig. 2).[24] These data strongly support the view that, with increasing sophistication of cellular coordination required to perform a function, the concentration of methylnitrosourea required to cause disruption of that function decreases. Since the EC_{50} (50% effective concentration; the concentration of methylnitrosourea that results in a 50% reduction in the test parameter) of methylnitrosourea for the immediate decrease in incorporation rates of precursors of macromolecular synthesis were similar to those for live birth rates,[24] it seems reasonable to presume that the effects of methylnitrosourea are immediate even if the manifestation of those effects may take place long after exposure (Table I). In aggregate these data suggest the possibility that blastocyst dysfunction is the result of subtle, nonlethal mutations that result in shifts in the developmental program of the embryo.

Table I. The 50% effective concentrations (EC_{50}) of methylnitrosourea

	EC_{50} (μg/ml)
Incorporation of:	
Tritium-labeled uridine	4.0,* 7.1†
Tritium-labeled thymidine	3.0,* 4.2† (4.2‡)
Tritium-labeled leucine	3.5*
Normal implantation	160†
Live birth	4.7

Modified from Iannaccone.[24]

*Incorporation rates determined at the end of a 1-hour exposure to methylnitrosourea.

†Incorporation rates determined 18 hours after a 1-hour exposure to methylnitrosourea.

‡Incorporation rates determined 18 hours after a 1-hour exposure of blastocysts without a zona pellucida to methylnitrosourea.

Indirect-acting mutagens. Similar embryonic dysfunction might be expected after exposure to indirect-acting mutagens (those that require metabolic activation in order to be mutagenic) if a relevant drug-metabolizing system capable of converting these chemicals to active metabolites were present in intimate contact with the developing embryo in the preimplantation period. Experiments were performed to determine if endometrial lining cells of the mouse uterus contain cytochrome P-450–dependent mixed function oxidase activity.[39] Cytochrome P-450 is known to be the enzyme system principally responsible for converting

stable polycyclic hydrocarbon procarcinogens to unstable electrophilic species capable of covalent interaction with deoxyribonucleic acid. The mouse uterine lining, consisting of both epithelial and stromal cells, is clearly a potential source of such enzymes. A number of stromal cell populations that are responsive to steroid hormones have been isolated.[40] Several cell strains have been isolated and are responsive to either estradiol and diethylstilbestrol or to progesterone. Other populations that are unresponsive to steroid hormones were isolated (Fig. 3). The progesterone-responsive cells can be growth inhibited by estrone or estriol. The cells contain inducible aryl hydrocarbon hydroxylase activity that catalyzes the conversion of benzo[a]pyrene to 3-hydroxy-benzo[a]pyrene and is one measure of cytochrome P-450 activity. Several clones of the endometrial stromal cell strains have been isolated in the presence or the absence of estradiol. These clones vary in response to estradiol with respect to cell growth.[40] Estradiol can reduce the basal and induced levels of aryl hydrocarbon hydroxylase activity when 2,3,7,8-tetrachlorodibenzo-p-dioxine is the inducer. The degree of induction of the activity by 2,3,7,8-tetrachlorodibenzo-p-dioxine is not afected by estradiol. The pattern of induction by benzanthracene in these clones is different.[40] The effect of hormone on aryl hydrocarbon hydroxylase induction by two different inducers implied that the inducers operate via separate mechanisms, presumably different aryl hydrocarbon receptors.[41, 42] This modulation of cytochrome P-450 activity by estrogen is associated with alterations in the pattern of microsomal proteins as revealed by denaturing sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Electrophoresis has also shown that induction in the mouse liver occurs via different isozymes than induction in endometrium and that benzanthracene and 2,3,7,8-tetrachlorodibenzo-p-dioxine induce different forms of cytochrome P-450 in the endometrial cells. Additionally, there is a protein with a relative molecular weight of 52,400 that, while induced by benzanthracene in the absence of estradiol, is not induced in its presence. There are several possible explanations: that the induction of cytochrome P-450 activity by benzanthracene is altered in some cells by estrogen as the result of an interaction between their activated receptors, which results in a different pattern of isozyme induction; that the level of gene expression of the relevant forms of cytochrome P-450 is directly effected by estrogen; or that the induced protein(s) is(are) modified posttranscriptionally in the presence of estrogen. A similar result has been obtained for the induction of uterine aromatase that can be modified by the presence of steroid.[43]

Endometrial stromal cells possess the ability to convert benzo[a]pyrene to active metabolites, including

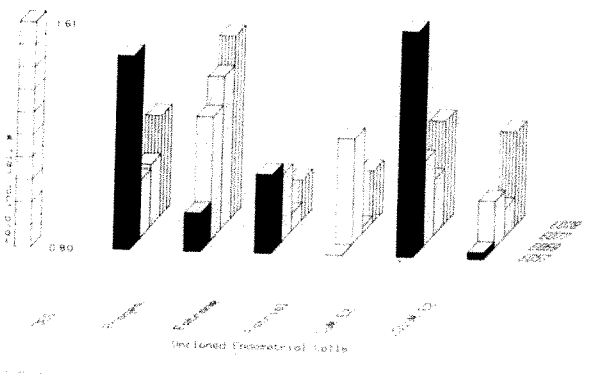


Fig. 3. Response of uncloned endometrial stromal cell strains to various steroids. The cell strain is shown on the z axis; the fold increase in cell number (in comparison to solvent-exposed cells) is shown on the y axis. Diethylstibestrol *(DES)*, progestrone *(Proges.)*, estrone, estriol, and 17β-estradiol *(E2)* were tested. (Reprinted with permission of Alan R. Liss, Inc. From Stols L, Iannaccone PM. Pattern of steroid interaction with inducers of aryl hydrocarbon hydroxylase activity in several cloned estradiol-responsive stromal cell strains from mouse endometrium. J Cell Physiol 1985;123:395-400.)

anti- and *syn*-benzo[a]pyrene-7,8-dihydrodiol 9,10-epoxides, which are capable of disrupting blastocyst functions in a concentration-dependent manner.[40] High-pressure liquid chromatography was used to demonstrate the site specificity of oxidation by cytochrome P-450 in microsomes isolated from estradiol-responsive mouse uterine cells.[40] Induced microsomes from mouse endometrial stromal cells were incubated with tritium-labeled benzo[a]pyrene and the metabolites were separated with high-pressure liquid chromatography. The resulting profiles demonstrated predominant oxidation at the 9,10 and 7,8 positions with no detectable 4,5 oxidation. These results support the conclusion that these cells are capable of generating benzo[a]pyrene-diol epoxides from benzo[a]pyrene-dihydrodiol. When microsomes from endometrial cells and blastocysts were incubated with benzo[a]pyrene-7,8-dihydrodiol for 1 hour, there were no morphologic effects, either immediately after exposure or 18 hours after exposure. This was true whether benzo[a]pyrene or the metabolite anti-benzo[a]pyrene-7,8-dihydrodiol 9,10-epoxide was used. Nevertheless, there was a concentration-dependent decrease in the frequency of total implantation and in the frequency of normal implantation rate, as well as in the number of animals that showed evidence of pregnancy when treated embryos were transferred to surrogate mothers. When embryos exposed in this manner were transferred to surrogates and allowed to come to term, a dose-dependent decrease in live birth rate was demonstrated.

The metabolite specificity of this effect was determined by exposing embryos to a racemic mixture

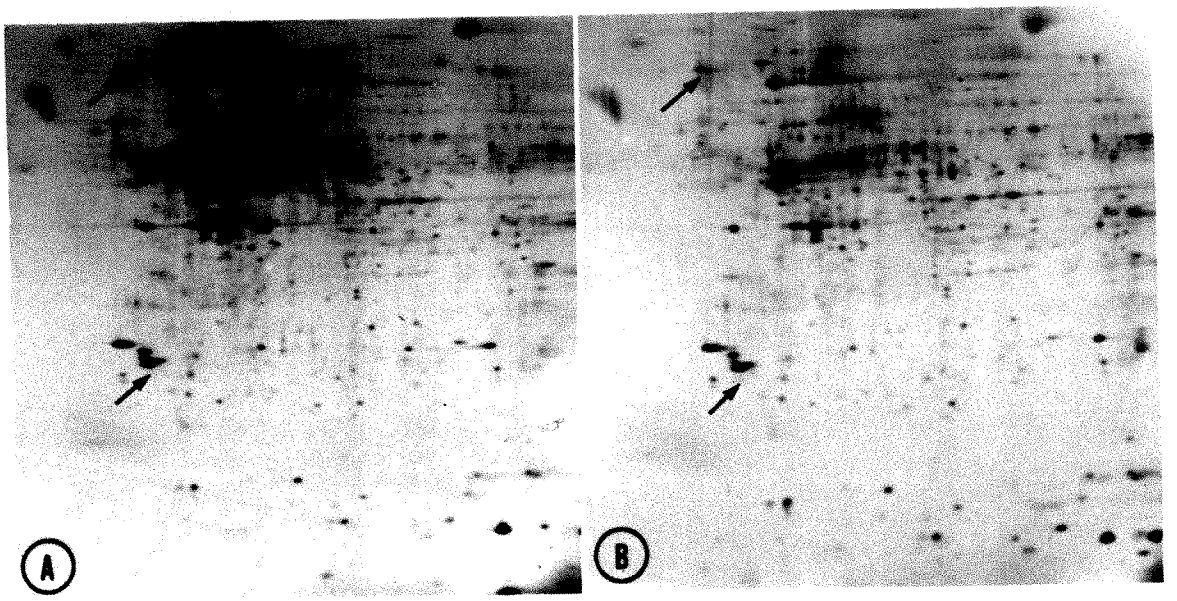


Fig. 4. Two-dimensional electrophoretogram of total protein from day 12 inbred mouse fetuses derived from blastocysts exposed in vitro to methylnitrosourea (10 μg/ml) with subsequent transfer to surrogate mothers. The second-dimension gels (shown here) have been silver stained and resolve approximately 900 spots. Panel *B* is from the fetus derived from methylnitrosourea-exposed blastocyst and Panel *A* is from the control (solvent-exposed) blastocyst. Two examples of protein alterations associated with treatment are illustrated *(arrows)*.

of *anti*-benzo[a]pyrene-7,8-dihydrodiol 9,10-epoxides. After insertion of embryos into the uteri of surrogates, there was a striking dose-dependent decrease in implantation rate and birth rate as well as a dose-dependent increase in the resorption rate. This particular metabolite is characterized by both cytotoxicity and mutagenicity in mammalian cells but not in bacteria. Similar experiments are being performed with *syn*-enantiomers, which are identical chemically except that the epoxide group is on the same side of the planar ring structure as the reference hydroxy-group. This difference, however, appears to profoundly decrease the mutagenicity of the metabolite while the cytotoxicity remains the same.[44]

Possible role of mutations

The possibility that induced mutation in the preimplantation period results in developmental dysfunction in the postimplantation period has been explored by attempts to identify altered proteins in very early organogenesis in the mouse. Embryos were exposed to two concentrations of methylnitrosourea at the blastocyst stage of development and reinserted into surrogate mothers. These pregnancies were interrupted at day 12, a time when organogenesis is just beginning in the mouse and maternal tissues can be critically separated from fetal tissues. The fetal tissues and fetal membranes were isolated and total protein preparations were subjected to two-dimensional electrophoresis (Fig. 4). The fetal tissues derived from methylnitrosourea-exposed blastocysts demonstrated a relatively small number of protein alterations. These alterations will be analyzed in an attempt to determine the role of mutagenesis in their formation as well as the role the alterations may play in poor pregnancy outcome.

It is clear that the coordinated expressions of the thousands of genes required to establish normal fetal development could not occur without orchestration by a relatively small set of supervisor or master genes. In *Drosophila* such genes have been identified and we now know that similar genes exist in mammalian species.[45-47] Whether these genes have the same function in Mammalia as they do in other animals remains to be established. We feel that the use of specific chemical disruption of embryonic function may serve as a valuable probe for the presence of such supervisor genes. Once the protein sequence of the altered protein is established, putative gene sequences can be deduced and used to define the presence and expression of the genes in normally developing fetal tissues.

The role of mutations in developmental defects can be addressed directly by analyzing insertional mutations that arise from the integration of exogenous deoxyribonucleic acid after pronuclear injection. Such transgenic animals have mutations wherever the exogenous deoxyribonucleic acid inserts in the host deoxyribonucleic acid. Occasionally, such a mutation occurs in a gene of developmental importance. If the transgenic animal is inbred to allow the expression of recessive phenotypes, then the role of the mutation in

the defect can be established. This has been accomplished in several instances. Insertional mutation of the α1(I) collagen gene results in disruption of blood vessel development and intrauterine death.[18] In the other example microinjection of deoxyribonucleic acid in pronuclear stage embryos resulted in a mutation of a gene that controls limb formation *(1d)*. Homozygous offspring of these animals suffer synostosis of the radius and the ulna, of the tibia and the fibia, as well as oligodactyly and syndactyly of digits in the forepaw and hindpaw.[19] Appropriate molecular probes are being used to sequence the genes responsible for these malformations.

In conclusion, broad advances in many other areas of biomedical research with attendant alleviation of human loss and suffering only serve to underscore our comparative inability to treat or even to understand birth defects and perinatal mortality. This must be due in large measure to our ignorance of the elegant mechanisms that organize and control fetal development in mammals. One can but hope that this situation will begin to change in the near future as new techniques are brought to bear on problems in reproductive biology.

REFERENCES

1. Simpson WJ. A preliminary report on cigarette smoking and the incidence of prematurity. AM J OBSTET GYNECOL 1957;73:808-15.
2. Hasselmeyer EG, Meyer MB, Cata C, Longo LD. In: Pinney JM, ed. Smoking and health: a report of the Surgeon General. Washington DC: US Government Printing Office, 1979:1-93.
3. Nasrat HA, Al-Hachim GM, Mahmood FA. Perinatal effects of nicotine. Biol Neonate 1986;49:8-14.
4. Weisberg E. Smoking and reproductive health. Clin Reprod Fertil 1985;3:175-86.
5. Castonguay A, Tjalve H, Trushin N, Hecht SS. Perinatal metabolism of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in C57BL mice. JNCI 1984;72:1117-24.
6. Wilson JG. Embryological considerations in teratology. In: Wilson JG, Warkany J, eds. Teratology: principles and techniques. Chicago: University of Chicago Press, 1965: 251-61.
7. Austin CR. Embryo transfer and sensitivity to teratogenesis. Nature 1973;244:333-4.
8. Tarkowski AK, Wroblewska J. Development of blastomeres of mouse eggs isolated at the 4- and 8-cell stage. J Embryol Exp Morphol 1967;18:155-80.
9. Rossant J, Vijh KM. Ability of outside cells from preimplantation mouse embryos to form inner cell mass derivatives. Dev Biol 1980;76:475-82.
10. Sutherland AE, Calarco-Gillam PG. Analysis of compaction in the preimplantation mouse embryo. Dev Biol 1983;100:328-38.
11. Lo C, Gilula NB. Gap junctional communication in the preimplantation mouse embryo. Cell 1979;18: 399-409.
12. McLachlin JR, Caveney S, Kidder GM. Control of gap junction formation in early mouse embryos. Dev Biol 1983;98:155-64.
13. Hillman N, Sherman MI, Graham C. The effect of spatial arrangement on cell determination during mouse development. J Embryol Exp Morphol 1972;28:263-78.
14. Dodd M, Andrew TA, Coles JS. Functional behaviour of skin allografts transplanted to rabbit deciduomata. J Anat 1980;130:381-90.
15. Slapsys RM, Clark DA. Active suppression of host-vs-graft reaction on pregnant mice. IV. Local suppressor cells in decidua and uterine blood. J Reprod Immunol 1982; 4:355-64.
16. Tyson JE, Mowat GS, McCoshen JA. Simulation of a probable biologic action of decidual prolactin on fetal membranes. AM J OBSTET GYNECOL 1984;148:296-300.
17. Herz Z, Khan I, Jayatilak PG, Gibori G. Evidence for the secretion of decidual luteotropin: a prolactin-like hormone produced by rat decidual cells. Endocrinology 1986;118:2203-9.
18. Jayatilak PG, Glaser LA, Basuray R, Kelly PA, Gibori G. Identification and partial characterization of a prolactin-like hormone produced by rat decidual tissue. Proc Natl Acad Sci USA 1985;82:217-21.
19. Fabro S. Reproductive toxicology: state of the art. Am J Ind Med 1983;4:391-3.
20. Spielmann H, Eibs HG, Habenicht U, Jacob-Muller U. Studies on the action of drugs during the preimplantation period in laboratory animals. Exp Biol Med 1982;7: 162-9.
21. Lin TP, Monie IW. The development of mouse blastocysts injected with, or cultured in, trypan blue solution. J Reprod Fertil 1973;32:149-51.
22. Epstein CJ, Smith SA. Handling of actinomycin D by preimplantation mouse embryos. Exp Cell Res 1978; 111:117-26.
23. Matsumoto N, Spindle A. Sensitivity of early mouse embryos to methylmercury toxicity. Toxicol Appl Pharmacol 1982;64:108-17.
24. Iannaccone PM. Long-term effects of exposure to methylnitrosourea on blastocysts following transfer to surrogate female mice. Cancer Res 1984;44:2785-9.
25. Adams CE, Hay MF, Lutwak-Mann C. The action of various agents upon the rabbit embryo. J Embryol Exp Morphol 1961;9:468-91.
26. Murray FJ, Schwetz BA, McBride JG, Staples RE. Toxicity of inhaled chloroform in pregnant mice and their offspring. Toxicol Appl Pharmacol 1979;50:512-22.
27. Sunderman FWJ, Shen SK, Mitchell JM, Allpass PR, Damjanov I. Embryotoxicity and fetal toxicity of nickel in rats. Toxicol Appl Pharmacol 1978;43:381-90.
28. Storeng R, Jonsen J. Nickel toxicity in early embryogenesis in mice. Toxicology 1981;20:45-51.
29. Takeuchi I. Teratogenic effects of methylnitrosourea on pregnant mice before implantation. Experientia 1984; 40:879-81.
30. Eibs H-G, Spielmann H, Hagele M. Teratogenic effects of cyproterone acetate and medroxyprogesterone treatment during the pre- and postimplantation period of mouse embryos. I. Teratology. 1982;25:27-36.
31. Storeng R, Jonsen J. Inhibitory effect of ricin on the development of preimplantation mouse embryos. J Toxicol Environ Health 1983;12(2-3):193-202.
32. Spielmann H, Eibs H-G. Recent progress in teratology. A survey of methods for the study of drug actions during the preimplantation period. Arzneimittelforschung Drug Res 1978;28:1733-42.
33. Snow MHL, Tam PPL. Is compensatory growth a complicating factor in mouse teratology? Nature 1979;279: 555-7.
34. Markert CL, Peters RM. Manufactured hexaparental mice show that adults are derived from three embryonic cells. Science 1978;202:56-8.
35. Hoffmann D, Brunnemann KD, Adams JD, Hecht SS. Formation and analysis of N-nitrosamines in tobacco products and their endogenous formation in consumers. IARC Sci Publ 1984;57:743-62.
36. Hoffmann D, Hecht SS. Nicotine-derived N-nitrosamines and tobacco-related cancer: current status and future directions. Cancer Res 1985;45:935-44.

37. Iannaccone PM, Tsao TY, Stols L. Effects of mouse blastocysts of in vitro exposure to methylnitrosourea and 3-methylcholanthrene. Cancer Res 1982;42:864-8.
38. Bossert NL, Iannaccone PM. Midgestational abnormalities associated with in vitro preimplantation N-methyl-N-nitrosourea exposure with subsequent transfer to surrogate mothers. Proc Natl Acad Sci USA 1985;82:8757-61.
39. Iannaccone PM, Stols L, Hollenberg PF, Gurka DP. An estradiol-responsive mouse endometrial cell strain with inducible aryl hydrocarbon hydroxylase activity. J Cell Physiol 1983;116:227-35.
40. Iannaccone PM, Fahl WE, Stols L. Reproductive toxicity associated with endometrial cell mediated metabolism of benzo[a]pyrene: a combined *in vitro, in vivo* approach. Carcinogenesis 1984;5:1437-42.
41. Stols L, Iannaccone PM. Pattern of steroid interaction with inducers of aryl hydrocarbon hydroxylase activity in several cloned estradiol-responsive stromal cell strains from mouse endometrium. J Cell Physiol 1985;123:395-400.
42. Okey AB, Vella LM. Binding of 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-p-dioxin to a common Ah receptor site in mouse and rat hepatic cytosols. Br J Biochem 1982;127:39-47.
43. Tseng L. The effect of estradiol and progesterone on human endometrial aromatase activity in primary cell culture. Endocrinology 1984;115:833-5.
44. King HW, Brookes P. On the nature of the mutations induced by the diolepoxide of benzo[a]pyrene in mammalian cells. Carcinogenesis 1984;5:965-70.
45. Levine M, Hafen E, Garber RL, Gerhing WJ. Spatial distribution of *Antennapedia* transcripts during *Drosophila* development. EMBO J 1983;2:2037-46.
46. Manley JL, Levine MS. The homeo box and mammalian development. Cell 1985;43:1-2.
47. Harding K, Rushlow C, Doyle HJ, Hoey T, Levine M. Cross-regulatory interactions among pair-rule genes in *Drosophila*. Science 1986;233:953-9.
48. Hartung S, Jaenisch R, Breindl M. Retrovirus insertion inactivates mouse α1(I) collagen gene by blocking initiation of transcription. Nature 1986;320:365-7.
49. Woychik RP, Stewart TA, Davis LG, D'Eustachio P, Leder P. An inherited limb deformity created by insertional mutagenesis in a transgenic mouse. Nature 1985; 318:36-40.

SYMPOSIUM

Infection: A Complication of Obstetric and Gynecologic Surgery

F. Gary Cunningham, M.D., *Chairman*
Dallas, Texas

The following papers are based on selected presentations delivered at the symposium, "Infection: A Complication of Obstetric and Gynecologic Surgery," held at Napa, California, July 24-27, 1986.

Funds for the symposium were provided by an educational grant from Lederle Laboratories.

Treatment and prevention of female pelvic infection: The quest for single-agent therapy

F. Gary Cunningham, M.D.
Dallas, Texas

The advantages of a single-agent regimen for prevention or treatment of female pelvic infections are outlined, and the characteristics of an "ideal" single agent are described. Indications for antimicrobial use in obstetrics and gynecology as well as the pathogens most likely to cause infection are reviewed. Empirical treatment regimens are assessed not only for efficacy but also for safety, simplicity, cost, and potential for inducing bacterial resistance. Piperacillin, as an example of an antimicrobial that may be appropriate for single-agent use in female pelvic infections, is evaluated in depth. (AM J OBSTET GYNECOL 1987;157:485-8.)

Key words: Single-agent therapy, antimicrobial resistance, surgical infection

Female pelvic infections almost always involve anaerobes and aerobes that compose normal cervicovaginal flora. To ensure adequate antimicrobial coverage for all potential pathogens, standard therapy usually consists of two or more antibiotics given simultaneously. Although such conventional multiagent regimens have been able to eradicate most of these infections, the quest for a clinically effective single agent continues to be of primary interest. In fact, the attractiveness of such an antimicrobial has been significantly enhanced in recent years by considerations that include the cost of mixing and administering several drugs compared with the simplicity of a single-drug regimen.

The increased use of perioperative prophylaxis over the past 15 years has added new, and sometimes worrisome, dimensions to the problem of treating infection-related complications. The potential for inducing microbial resistance is integral to any decision regarding prophylactic antibiotic use. Thus ideal single-agent therapy would include minimal resistance induction.

From the Department of Obstetrics and Gynecology, University of Texas Health Science Center, Southwestern Medical School.

Ideal single agent

The ideal single antibiotic for treatment and prevention of female pelvic infection must have a wide spectrum of in vitro activity inclusive of most major pathogenic bacteria likely to cause such infections. Moreover, the agent should have a well-studied and verified record in clinical practice. It should also have suitable tissue penetration at target sites most likely to be involved, such as pelvic abscesses. The ideal agent would have minimal potential for inducing bacterial resistance, thus making it useful for perioperative prophylaxis as well as for treatment. Finally, it should be safe and relatively inexpensive.

Piperacillin is a semisynthetic acylamino penicillin that has most of these characteristics and is used as monotherapy for treatment and prevention of female pelvic infection. In this symposium, we will review the clinical aspects of piperacillin vis-à-vis specific infections that we frequently prevent or treat, and we may conclude that it fulfills many requirements of an ideal agent for single-drug therapy.

Antimicrobial indications

Nonsurgical pelvic infection. By far, the most common indications for parenteral antimicrobial use in obstetrics and gynecology are pelvic infections that occur after operation. Nevertheless, we do encounter nonsurgical infections, the most common of which is acute, community-acquired salpingitis. These infections can be complicated by peritonitis and tuboovarian abscesses and frequently cause sterility. Other nonsurgical infections include perineal and Bartholin gland abscesses, which can be particularly dangerous in women with diabetes.

Surgical infections. Pelvic infections that occur after obstetric or gynecologic operations are most frequently encountered after cesarean section or abdominal or vaginal hysterectomy. Other surgical procedures, such as ovarian or tubal operations, are seldom complicated by infection and thus have not been extensively studied.

Prophylaxis. Although there are no rigid guidelines to define women who are candidates for perioperative prophylaxis, most infectious disease specialists agree that antimicrobials significantly reduce the rate of postoperative infection among women undergoing procedures known to be, or likely to be, contaminated. Specific indications for such prophylaxis in cesarean section or abdominal or vaginal hysterectomy will be addressed during this symposium.

Pathogens

Studies have shown that most female pelvic infections, surgical or nonsurgical, are caused by bacteria that compose the normal cervicovaginal flora. In 1977, for example, we reported a study of nearly 200 women with acute salpingitis and showed that the pathogens isolated from the peritoneal fluid taken from these women were identical to those found in women with postoperative obstetric and gynecologic infections.[1] Unlike surgical patients, these women apparently have a breach of the epithelial defenses caused by primary infection with pathogens that are not considered normal flora.

Sexually transmitted diseases: The exceptions. The most obvious exceptions with respect to the etiology of community-acquired salpingitis are the sexually transmitted pathogens *Neisseria gonorrhoeae, Chlamydia trachomatis,* and perhaps others. In our 1977 study, we found that about half of the cases were initiated by gonococcal infection, and Scandinavian investigators have subsequently concluded that nearly one third of cases of salpingitis are initiated by *C. trachomatis* infection. This incidence is probably similar in American women. However, in as many as half of women with salpingitis, initial infection with *N. gonorrhoeae* or *C. trachomatis* is followed by secondary infection with opportunistic organisms that compose the normal flora, and thus they are similar to postoperative infections.

Bacterial isolates. In general, bacteria most commonly isolated from women with pelvic infections are anaerobic streptococci (*Peptostreptococcus* and *Peptococcus* sp.), aerobic streptococci (group B streptococci and enterococci), coliforms (*Escherichia coli* and *Klebsiella*), *Bacteroides* sp. (*B. fragilis* and *B. bivius*), and *Clostridium* sp. Some of these deserve special mention.

Bacteroides. Approximately 10% to 20% of women with pelvic infections are infected with a member of the genus *Bacteroides (B. fragilis, B. bivius, B. melaninogenicus),* and this remains an important consideration for selecting an antimicrobial regimen for treatment. For perioperative prophylaxis, activity against *Bacteroides* and specifically *B. fragilis* appears less important.

We must continue to carefully monitor susceptibility of *B. fragilis* because of its pathogenicity and typical antimicrobial resistance. During the mid-1960s, for example, *Bacteroides*—a relatively "new" discovery at the time—was quite sensitive to the tetracyclines, but currently as many as 65% of isolates are resistant. Without in vitro susceptibility audits, however, we would have experienced 2 to 3 years of clinical failures and morbidity before arriving at the conclusion that, because of resistance by *Bacteroides,* tetracycline was no longer effective as empirical therapy for pelvic infection caused by these organisms.

Enterococci. After anaerobic streptococci, the pathogens most frequently isolated from women with pelvic infection at Parkland Hospital are aerobic streptococci. Particularly worrisome is that enterococci *(Streptococcus faecalis)* are isolated from nearly 25% of these women when cultures are taken from the uterus, amniotic fluid, or vaginal cuff. The pathogenicity of enterococci, which are generally considered low-virulence organisms, is still a matter of controversy. Nevertheless, since the organism is highly resistant to most currently used antimicrobials, a growing number of clinicians are intuitively concerned about providing coverage for it. Microbiologic trends indicate that enterococci are isolated more often than in previous decades, and there is concern that they may prove to be the emerging pathogens—the methicillin-resistant *Staphylococcus*—of the 1980 and 1990s.

Resistance

The problem of bacterial resistance to many potent antimicrobials is no longer theoretical and is a serious

threat to effective treatment of infection. Therapy for most surgical infections is empirical, and we seldom prescribe an antimicrobial on the basis of culture results. Audits of bacterial susceptibilities are crucial therefore to continuing successful empirical therapy by allowing detection of ecologic shifts and acquired resistance of bacterial strains to standard therapeutic regimens.

Resistance of *Bacteroides* has been reported with increasing frequency in recent years and is a problem of particular concern to those interested in obstetric and gynecologic infections. Cuchural et al.[2] examined patterns of resistance for 500 to 700 isolates each year for 1981 and 1982 and reported a rise in the incidence of resistance of *Bacteroides* strains to cefoxitin in vitro from 8% to 16%. This also reflects our experience with clinical isolates from women with post–cesarean section infections at Parkland Hospital.[3] There have been disturbing reports of in vitro resistance of *Bacteroides* to clindamycin, the "gold standard," as high as 10% in some centers since 1980.[4] Although clinical failures have not as yet reflected such in vitro resistance, it is imperative that we be aware of these trends to continue rational selection of empirical regimens.

In surgical prophylaxis, not only is existing bacterial resistance important but also there is concern about inducing resistance. Although it is uncertain whether such limited antimicrobial exposure induces significant resistance, antibiotic pressure in the form of numerous exposures definitely selects out nonsusceptible organisms. Another consideration is whether the drugs given for surgical prophylaxis induce β-lactamases that might render these agents useless in the future.

Empirical treatment regimens

Since treatment of female pelvic infections is largely empirical, antimicrobials used should be effective against the wide spectrum of potential pathogens. Although mild-to-moderate pelvic infections are frequently treated with a second-generation cephalosporin alone, serious infections are best treated with a "big gun" regimen. For clinical purposes, clindamycin plus an aminoglycoside remains the "gold standard" in terms of providing the best coverage for empirical treatment.[5] Although highly effective, this regimen has drawbacks that include toxicities associated with the aminoglycoside as well as costs of extra staff time required to administer and monitor two drugs. To avoid the side effects of aminoglycosides, some practitioners prefer clindamycin plus a cephalosporin. With this regimen, toxicity is less of a concern, but the expense of a two-drug regimen remains. Another highly effective regimen, used infrequently, is the combination of a cephalosporin or penicillin with chloramphenicol. Many feel that the rare possibility of chloramphenicol-induced aplastic anemia precludes its use. Finally, metronidazole, which has excellent in vitro activity against anaerobes, has been used in combination with an aminoglycoside or a penicillin; however, the clinical efficacy of such regimens has not been tested extensively.

Cefoxitin, a second-generation cephamycin, is frequently used as monotherapy for mild-to-moderate infection possibly caused by *B. fragilis*. As with other regimens, enterococci are not covered, but the consequences of this window in its spectrum are uncertain. Cefoxitin induces β-lactamases, which may be of clinical importance.

Piperacillin: single-agent therapy?

The suitability of any antimicrobial for single-agent therapy depends on how well it fulfills criteria previously outlined. Piperacillin is highly effective in vitro against both anaerobes and aerobes commonly isolated from female pelvic infections.[4] In a survey of 1140 mixed isolates from women undergoing hysterectomy at Parkland Hospital, Heard et al.[5] reported that piperacillin had the greatest in vitro activity of 13 drugs tested. An important exception to coverage obtained with piperacillin, as with all β-lactams, is lack of activity against *C. trachomatis*. This intracellular organism is associated with 30% to 50% of cases of acute salpingitis in the United States, and specific therapy with a tetracycline or erythromycin is used to eradicate it. A less important window with respect to antimicrobials given for pelvic infections is the lack of activity against coagulase-positive β-lactamase–producing staphylococci.

Enterococcal coverage. Since the pathogenicity of enterococci is controversial, the efficacy of piperacillin against these aerobic streptococci is currently a theoretical advantage. We continue to isolate these organisms from 25% of women with pelvic infections at Parkland, and in light of recent microbiologic trends, enterococcal coverage may prove to be a pragmatic, and crucial, advantage in the future.

Clinical efficacy. Piperacillin has been shown clinically effective in preliminary studies reported thus far. Since 1983, Sweet and Gilstrap and their colleagues[6,7] have reported on a total of 65 women with moderately severe pelvic infections that were treated with piperacillin. These were randomized studies and the comparative drugs were cefoxitin or clindamycin plus gentamicin. There was a 92% clinical success rate with piperacillin, and side effects were negligible. The results of other studies will be reported in this symposium.

β-Lactamase induction. Piperacillin is relatively resistant to most β-lactamases and does not readily induce microbial β-lactamase production. Thus bacterial resistance to piperacillin is less common than with "high inducers" of β-lactamases, such as some of the cephalosporins. Since perioperative antimicrobial prophylaxis is commonplace in numerous surgical procedures,

agents that do not induce bacterial resistance may be particularly valuable. Moreover, single-dose prophylaxis would intuitively lower the potential for resistance even further, and the efficacy of such regimens is being studied.

Safety. Piperacillin enjoys minimal toxicity common to all penicillins and can be used with confidence in nonallergic patients. Unlike aminoglycosides, piperacillin is not usually nephrotoxic and is not ototoxic.

Cost. There are several ways in which drug costs are assessed. The simplest is the basic cost of a drug itself, which, for piperacillin, is relatively low compared with the cost of some third-generation cephalosporins. Another way is to calculate staff costs for mixing, administering, and monitoring, and single-agent therapy is almost always less expensive than combination regimens. Finally, the risk and inherent costs of antibiotic failure can be high and can render low-priced but less effective regimens more expensive.

REFERENCES

1. Cunningham FG, Hauth JC, Strong JD, et al. Evaluation of tetracycline or penicillin and ampicillin for treatment of acute pelvic inflammatory disease. N Engl J Med 1977; 296:1380.
2. Cuchural GJ, Tally FP, Jacobus NV, et al. Antimicrobial susceptibilities of 1,292 isolates of the *Bacteroides fragilis* group in the United States: comparison of 1981 with 1982. Antimicrob Agents Chemother 1984;26:145.
3. Heard ML, Bawdon RE, Hemsell DL, Nobles BJ. Susceptibility profiles of potential aerobic and anaerobic pathogens isolated from hysterectomy patients. AM J OBSTET GYNECOL 1984;149:133.
4. Tally FP, Cuchural GJ, Jacobus NV, et al. Nationwide study of the susceptibility of the *Bacteroides fragilis* group in the United States. Antimicrob Agents Chemother 1985;28: 675.
5. DiZerega G, Yonekura L, Roy S, Nakamura RM, Ledger WJ. A comparison of clindamycin-gentamicin and penicillin-gentamicin in the treatment of post–cesarean section endomyometritis. AM J OBSTET GYNECOL 1979;134: 238.
6. Sweet RL, Robbie MO, Ohm-Smith M, Hadley WK. Comparative study of piperacillin versus cefoxitin in the treatment of obstetric and gynecologic infections. AM J OBSTET GYNECOL 1983;145:342.
7. Gilstrap LC III, Maier RC, Gibbs RS, Connor KD, St Clair PJ. Piperacillin versus clindamycin plus gentamicin for pelvic infections. Obstet Gynecol 1984;64:762.

Single-agent therapy for women with acute polymicrobial pelvic infections

David L. Hemsell, M.D., Molly C. Heard, R.A., Brenda J. Nobles, and Patricia G. Hemsell, R.A.

Dallas, Texas

Pathogens that cause acute polymicrobial female pelvic infections usually do not differ from those that compose the normal flora of the lower reproductive tract. Accurate identification of these bacteria is difficult because cultures obtained via the lower tract can easily be contaminated. Although use of a double-lumen catheter-protected brush culture cannot completely eliminate the risk of contamination, it is the least invasive method for obtaining culture material from the upper reproductive tract. Compounding the problem of accurately identifying pathogens that cause acute upper tract infections is the fact that bacteria appear to be present in the upper tracts of asymptomatic women with normal examinations. Because of these problems and because of the polymicrobial nature of these infections, empiric therapy frequently includes more than one antimicrobial agent. Newer, semisynthetic penicillins and cephalosporins have expanded spectrums of in vitro activity against most of the bacteria frequently recovered from pelvic infection sites. Comparative clinical trials have shown these agents to be as effective when used alone as is combination therapy. With few exceptions, empiric monotherapy with one of these newer antimicrobials will be curative for women with acute upper tract infection, will have less potential toxicity, will require less space, materials, and manpower to administer, and will be less expensive. (AM J OBSTET GYNECOL 1987;157: 488-90.)

Key words: Polymicrobial, flora, aminoglycoside, expanded-spectrum penicillins, cephalosporins

From the Department of Obstetrics and Gynecology, University of Texas Health Science Center at Dallas, Southwestern Medical

Reprint requests: David L. Hemsell, M.D., Department of Obstetrics and Gynecology, University of Texas Health Science Center, South-

The normal flora of the lower reproductive tract has been described by numerous investigators.[1-5] It is from these bacteria that inoculation of a pelvic operative site occurs, resulting in hospital-acquired infection after hysterectomy or cesarean section. The same pathogens may be recovered from women with community-acquired infection. *Neisseria gonorrhoeae* is isolated from the majority of women treated in the United States for acute salpingitis. A significant problem for the obstetrician and gynecologist is how to accurately culture the infected pelvic site. Modification of a bronchial double-lumen catheter brush device now allows culture of the endometrium with little, if any, contamination by lower reproductive tract flora.[6] It is much more difficult to culture the infection site in women who develop a pelvic infection after hysterectomy because the infection is usually cephalad and lateral to the vaginal surgical margin. When an accurate culture is performed, it is not uncommon to recover a mixture of three to nine aerobic and anaerobic gram-positive and gram-negative bacteria from infection sites, compounding the problem of accurately identifying the true pathogen. Although blood cultures are quite accurate, most are sterile prior to treatment for acute pelvic infection.

Upper tract flora

Ongoing research in our institution indicating that there may be a "normal flora" in the upper reproductive tract may further compound the problem of accurately identifying pathogens in women with acute community-acquired pelvic infection. Women recently admitted for treatment of acute pelvic inflammatory disease had endometrial cultures performed with the protected-brush device prior to treatment as well as when they attended the clinic 4 to 14 days after therapy. Even though they were afebrile, asymptomatic, and had a normal pelvic examination, a mean of more than three bacteria was recovered from protected endometrial cultures. Gram-positive aerobes and gram-positive and gram-negative anaerobes including *Bacteroides fragilis* were recovered, as was *Chlamydia trachomatis* (unpublished data). Those unsuspected results stimulated similar evaluations in consenting asymptomatic women with normal pelvic examinations who were attending our family planning clinics for annual examinations. None had a history of prior acute pelvic inflammatory disease. A mean of more than four bacteria was recovered from 36 women for whom such data are available. This appeared to confirm the hypothesis that an upper reproductive tract flora may exist. Peritoneal fluid from asymptomatic women undergoing elective tubal sterilization has been cultured and found to contain bacteria.[7]

Combination therapy

The polymicrobial nature of acute pelvic infections has been thoroughly elucidated.[8-11] As a result, combination antimicrobial therapy was usually used for initial empirical therapy of such infections. Penicillin, for example, was usually combined with an aminoglycoside or tetracycline, with an efficacy of 70% to 80%. Results with such regimens were similar in our hospital.[12] An anaerobic-specific antimicrobial such as clindamycin or chloramphenicol was not included initially because of the potential toxicity associated with these agents. Since a third antibiotic was required in 20% to 30% of cases, and since a significant number of anaerobes was recovered from cultures taken from pelvic infection sites, a decision was made to evaluate anaerobic-specific antimicrobials as part of initial therapy. Efficacy with clindamycin/gentamicin was 95% compared with only 71% for penicillin/gentamicin, clearly indicating a need to include predictable antianaerobic activity as part of initial therapy.[13] Both clindamycin and metronidazole have been combined with an aminoglycoside and are associated with efficacy of 95% or better.[14]

Many obstetricians and gynecologists administered ampicillin as initial therapy for pelvic infections and continue to do so with success (~95% efficacy). If such is the case, alteration in therapeutic regimen is not indicated. However, if the result is comparable with the 81% efficacy rate reported by Sorrell et al.,[15] altering the therapeutic regimen should be considered to achieve and maintain efficacy in the mid-90% range. Newer semisynthetic penicillins have an expanded spectrum that includes bactericidal activity against gram-positive and gram-negative aerobic and anaerobic bacteria.[15-17] The same is true for expanded-spectrum cephalosporins.[16, 13-20]

Aminoglycosides

With the development of synthetic antimicrobials that demonstrate in vitro activity against a wide range of gram-positive and gram-negative aerobic and anaerobic bacteria and that are as effective clinically as combination therapy, the necessity for combination therapy becomes less clear. Most combination regimens include an aminoglycoside. The predominant gram-negative aerobic pathogen in pelvic infections is *Escherichia coli*. Although *E. coli* and other Enterobacteriaceae recovered from women with acute pelvic infection are usually exquisitely sensitive to aminoglycosides, they are also sensitive to many of the newer penicillins and cephalosporins. The therapeutic range for gentamicin falls between peak concentrations of 5.0 and 8.0 μg/ml and trough concentrations of 0.5 and 1.8 μg/ml at an 8-hour dosing interval. Renal toxicity and ototoxicity occur in 3% to 10% of patients treated with aminoglycosides and usually occur when peak concentrations are >12 to 15 μg/ml and trough concentrations exceed 2 μg/ml. To make these determinations, repeated serum gentamicin concentrations must be performed. Since each such determination costs approximately $30, using serum concentrations to adjust dos-

age can contribute significantly to higher or increased health care costs. If such precision therapy is required to eradicate a pathogen sensitive only to aminoglycosides, the cost would certainly be justified. Fortunately, however, such a situation is rare in acute pelvic infections.

The recommended gentamicin dose for patients with severe infections is 3.5 mg/kg/day and is increased to 5 mg/kg/day in life-threatening infections. Zaske et al.[21, 22] have determined the pharmacodynamics of gentamicin in both obstetric and gynecologic patients and reported that the dose required for the treatment of such patients with acute pelvic infection ranges from 1.9 to 14.0 mg/kg/day. In obstetric patients and gynecologic patients, respectively, mean doses of 5.2 and 5.1 mg/kg/day were required to maintain the concentration of gentamicin within a therapeutic range. Forty-seven percent of the women required more than 5 mg/kg/day to maintain therapeutic concentrations. It was also learned that approximately 75% of patients required a 6-hour—not an 8-hour—dosing interval. Thus combination therapy that includes an aminoglycoside is more costly, is potentially more toxic, requires monitoring, and requires more man hours to accomplish.

Single-agent therapy

The primary concern of the physician treating a woman with an acute polymicrobial soft tissue pelvic infection obviously is curing the infection. If various regimens are therapeutically equivalent, the potential for toxicity or adverse reaction should be the next consideration. Finally, if regimens are relatively equivalent with respect to efficacy and safety, cost becomes the important consideration. Regimens combining several therapeutic agents are occasionally necessary in the treatment of the woman with an acute pelvic infection but should demonstrate superior efficacy to justify the risks of adverse reactions and the increased costs of drug, storage space, and manpower associated with their use. Such justification exists in infections in which a potential pathogen is identified against which the single-agent regimen is ineffective, such as *C. trachomatis* in a woman with acute salpingitis. Large tuboovarian or pelvic abscesses are in another category of infection for which combination therapy is appropriate. Single-agent therapy with newer expanded-spectrum penicillins and cephalosporins has been repeatedly shown to be equally as effective as combination therapy for most types of pelvic infection.

REFERENCES

1. Gorbach SL, Menda KB, Thadepalli H, Keith L. Anaerobic microflora of the cervix in healthy women. Am J Obstet Gynecol 1973;117:1053-5.
2. Tashjian JH, Coulam CB, Washington JA II. Vaginal flora in asymptomatic women. Mayo Clin Proc 1976;51:557-61.
3. Levison ME, Corman LC, Carrington ER, Kaye D. Quantitative microflora of the vagina. Am J Obstet Gynecol 1977;127:80-5.
4. Bartlett JG, Moon NE, Goldstein PR, Goren B, Onderdonk AB, Polk BF. Cervical and vaginal bacterial flora: ecologic niches in the female lower genital tract. Am J Obstet Gynecol 1978;130:658-61.
5. Spence M, Blanco L, Patel J, Brokman M. A comparative evaluation of vaginal, cervical, and peritoneal flora in normal, healthy women: a preliminary report. Sex Transm Dis 1982;9:37-40.
6. Knuppel RA, Scerbo JC, Dzirik J, Mitchell GW Jr, Cetrulo CL, Bartlett J. Quantitative transcervical uterine cultures with a new device. Obstet Gynecol 1981;57:243-8.
7. Cunningham FG, Hauth JC, Gilstrap LC, Herbert WNP, Kappus SS. The bacterial pathogenesis of acute pelvic inflammatory disease. Obstet Gynecol 1978;52:161-4.
8. Swenson RM, Michaelson TC, Daly MJ, Spaulding EH. Anaerobic bacterial infections of the female genital tract. Obstet Gynecol 1973;42:538-41.
9. Sweet RL. Anaerobic infections of the female genital tract. Am J Obstet Gynecol 1975;122:891-901.
10. Bartlett JG. Anaerobic infections of the pelvis. Clin Obstet Gynecol 1979;22:351-60.
11. Gilstrap LC III, Cunningham FG. The bacterial pathogenesis of infection following cesarean section. Obstet Gynecol 1979;53:545-9.
12. Hemsell DL, Cunningham FG. Combination antimicrobial therapy for serious gynecologic and obstetric infections—obsolete? Clin Ther 1981;4(suppl A):81-8.
13. diZerega G, Yonekura L, Roy S, Nakamura RM, Ledger WJ. A comparison of clindamycin-gentamicin and penicillin-gentamicin in the treatment of post–cesarean section endomyometritis. Am J Obstet Gynecol 1979; 134:238-42.
14. Gall SA, Kohan AP, Ayers OM, Hughes CE, Addison WA, Hill GB. Intravenous metronidazole or clindamycin with tobramycin for therapy of pelvic infections. Obstet Gynecol 1981;57:51-8.
15. Sorrell TC, Marshall JR, Yoshimori R, Chow AW. Antimicrobial therapy of postpartum endomyometritis. II. Prospective, randomized trial of mezlocillin versus ampicillin. Am J Obstet Gynecol 1981;141:246-51.
16. Sweet RL, Robbie MO, Ohm-Smith M, Hadley WK. Comparative study of piperacillin versus cefoxitin in the treatment of obstetric and gynecologic infections. Am J Obstet Gynecol 1983;145:342-9.
17. Gilstrap LC III, Maier RC, Gibbs RS, Connor KD, St Clair PJ. Piperacillin versus clindamycin plus gentamicin for pelvic infections. Obstet Gynecol 1984;64:762-6.
18. Cunningham FG, Hemsell DL, DePalma RT, Kappus S, Roark M, Nobles B. Moxalactam for obstetric and gynecologic infections; in vitro and dose-finding studies. Am J Obstet Gynecol 1981;139:915-21.
19. Gibbs RS, Blanco JD, Duff P, Castaneda YS, St Clair PJ. A double-blind, randomized comparison of moxalactam versus clindamycin-gentamicin in treatment of endomyometritis after cesarean section delivery. Am J Obstet Gynecol 1983;146:769-72.
20. Hemsell DL, Cunningham FG, De Palma RT, Nobles BJ, Heard M, Hemsell PG. Cefotaxime sodium therapy for endomyometritis following cesarean section: dose-finding and comparative studies. Obstet Gynecol 1983;62:489-97.
21. Zaske DE, Cipolle RJ, Strate RG, Malo JW, Koszalka MF Jr. Rapid gentamicin elimination in obstetric patients. Obstet Gynecol 1980;56:559-64.
22. Zaske DE, Cipolle RJ, Strate RG, Dickes WF. Increased gentamicin dosage requirements: rapid elimination in 249 gynecology patients. Am J Obstet Gynecol 1981;139: 896-900.

Changing concepts in obstetric antibiotic prophylaxis

Rudolph P. Galask, M.D.
Iowa City, Iowa

The evolution of antibiotic prophylaxis in cesarean section is traced from the discovery of sulfa compounds in the 1940s to the present. Prolonged courses of antibiotics, initiated before the surgical incision, have given way to shorter, three-dose and even single-dose regimens administered after clamping of the umbilical cord. Several factors have been proposed to help identify patients at greatest risk of infection. Guidelines for antibiotic prophylaxis are reviewed. The effects of antibiotics on host flora are described, and the implications of bacterial resistance for selection of a prophylactic agent, particularly with respect to induction of the Richmond-Sykes type I β-lactamase enzyme, are discussed. (Am J Obstet Gynecol 1987;157:491-7.)

Key words: Perioperative antibiotic prophylaxis, β-lactamase enzymes

A major challenge for physicians dealing with parturients has been the potential occurrence of serious infections during the postpartum period. The incidence of infection is of even greater concern for those patients who undergo an operative delivery. Because of these issues, early attempts to decrease the incidence of infections with prophylactic antibiotics were reported soon after effective drugs were discovered. In 1943, Richards,[1] for example, reported that the use of sulfa compounds decreased morbidity. Hesseltine and Thelen,[2] however, using local applications of sulfas under the bladder flap in 1948, did not appreciably reduce the infection rate and suggested that local prophylaxis of this type would result in a marked increase in adhesions at the time of a subsequent operation. They concluded therefore that the use of these compounds was not beneficial to their patients. Keettel and Plass[3] studied the use of intramuscular penicillin given to 382 women delivered vaginally and compared the incidence of infections in this group with that in 391 untreated control patients. They reported infection rates of 6.4% in the control group and 1.3% in the treated group and observed that the number of gram-positive organisms decreased while the gram-negative organisms increased in the treated group when compared with the control group. Although a lower incidence of infections in the treated patients was demonstrated, Keettel and Plass concluded that the penicillins might be more useful as prophylaxis in patients requiring a cesarean section. They did not, however, study that population.

Because of questions regarding the possible development of microbial resistance, the use of prophylactically administered antibiotics was not well accepted in obstetric practice during the 1950s and 1960s. Indeed the efficacy of antibiotic prophylaxis remained controversial among all of the surgical specialties. In a paper presented at the Twenty-second Annual Meeting of the Society of University Surgeons, Burke[4] reported results of a study that delineated a specific period during which antibiotic action could effectively prevent staphylococcal wound infections in an experimental animal model. He was able to show that if an antibiotic was given at any time from 1 hour prior to the inoculation of organisms up to 3 hours after the insult, a significant reduction in wound size occurred. This study, which was later published, established the currently recognized principle on which effective use of antibiotic prophylaxis is based, namely, that adequate levels of the antimicrobial agent must be in the tissue at the time of contamination.

During the 1970s, several changes in the practice of obstetrics increased our understanding of peripartum complications, particularly those involving sepsis. Improved microbiologic techniques, especially methods to isolate and identify anaerobic microorganisms, led to a clearer perception of the mechanisms of abscess formation. Obstetricians interested in infectious disease began to recognize the role of the indigenous microflora as the "contaminants" that caused the majority of postoperative infectious complications. Moreover, for a variety of reasons, the number of cesarean sections being performed was markedly increasing.[5] All of these factors contributed to the sense that the incidence of infection-related complications was unacceptably high and provided the impetus for the use of perioperative antibiotic administration in obstetrics.

Evolution of prophylactic regimens

Reports of sepsis in more than 60% of patients untreated after cesarean section compared with significantly fewer postoperative infections in treated patients

From the Department of Obstetrics and Gynecology, The University of Iowa College of Medicine.
Reprint requests: Rudolph P. Galask, M.D., Department of Obstetrics and Gynecology, The University of Iowa College of Medicine, Iowa City, IA 52242.

prompted a number of obstetrician investigators to recommend the use of antibiotic prophylaxis for patients undergoing this procedure.[6-10] Early studies used a prolonged regimen consisting of an initial dose prior to the incision followed by 3 to 5 days of antibiotic therapy.[5, 11-13] Fortunately, several investigators recognized early that such regimens were therapeutic rather than prophylactic and recommended shorter, 1-day, three-dose courses beginning prior to the procedure and followed by only two subsequent doses of drug. A major advance in obstetric prophylaxis was reported by Gordon et al.[14] when they showed that the administration of ampicillin *after* the umbilical cord was clamped was as effective as giving the drug prior to the operative delivery procedure. This logical finding, which agreed with Burke's previous work, eliminated the possibility of placental transfer of antibiotic to the fetus. Since a number of other reports confirmed that delaying administration of the antibiotic until termination of blood flow to the infant was as effective for prophylaxis as preoperative administration, the majority of obstetricians have accepted this practice. The use of multiple doses of antibiotic to the mother during the first postoperative day was, however, continued. In 1983, Hawrylyshyn et al.[15] reported a significant decrease in postoperative infections after a single, 2 gm dose of cefoxitin given at the time the umbilical cord was clamped. Even though this important finding has been confirmed by others, single-dose prophylaxis has not been universally adopted.[16-18]

Identifying patients at risk

Unfortunately, early studies included all patients undergoing cesarean section and did not identify the infectious sites that were responsible for the reported morbidity. When a number of investigators became concerned about the indiscriminate use of antibiotics in all patients, attempts were made to identify those patients who were at greatest risk and who would obtain the greatest benefit.

Gibbs and Weinstein[19] were the first investigators to report data regarding the risk of infection in their patient population. They found that at their institution, regardless of whether patients were undergoing primary or repeat cesarean section, the risk of infection when antibiotics were not used was high. Johnson et al.,[20] on the other hand, found that patients undergoing elective repeat cesarean section were at low risk of developing infections and did not benefit from antibiotic prophylaxis. Phelan and Pruyn[21] also reported that the risk of infection was greatest in those patients undergoing primary cesarean sections and that the use of an antibiotic resulted in substantially improved outcomes. Further refinements regarding the patient population at risk were reported by Rothbard et al.,[22] who demonstrated that women in labor prior to the procedure were at greater risk than those who were not in labor at the time of primary cesarean section. Others have reported similar findings.[10, 13, 14, 23]

In an attempt to further define the factors that place patients at risk for postoperative sepsis, Green et al.[8] analyzed 15 variables and showed that anemia and a general anesthetic, as well as labor, were risk factors in their patients. Other investigators have reported that ruptured membranes[10] and internal fetal monitoring[9] can also be considered factors that increase the risk of infection. The identification of the patient population at risk, as well as the various events that might predispose otherwise normal individuals to a septic postoperative course, is extremely important. If one could reasonably predict and selectively administer antibiotic prophylaxis only to the high-risk population, the majority of patients would be spared the potential consequences of an unnecessary drug. At present, it appears that women in labor with ruptured membranes who are undergoing a primary cesarean section are at risk of infection. The importance of such variables as low socioeconomic status, obesity, anemia, internal fetal monitoring, type of anesthesia, and blood loss as predisposing factors appears to vary from report to report. Therefore the significance of any or all of these confounding variables must be individualized and considered along with the infection rate at a given institution.

Selecting an agent

Since 1968, when Miller and Crichton[6] reported that ampicillin prophylaxis was effective in decreasing postoperative infections, an increasing number of drugs has been added to the list. In fact, to date, penicillin, ampicillin, carbenicillin, ticarcillin, piperacillin, cephalothin, cephaloridine, cefazolin, cefamandole, cefoxitin, cephradine, cefoperazone, cefotaxime, moxalactam, cefotetan, kanamycin, gentamicin, clindamycin, metronidazole, and sulfamethoxazole-trimethoprim have been used for surgical prophylaxis, and all have demonstrated efficacy either alone or in combination with another compound. The difficulty in comparing drug efficacies is that these studies varied with respect to patient populations, time, duration, route of administration, and morbidity criteria, as well as major bias potential due to lack of prospective blinding of the subjects and controls. Nevertheless, when one considers the studies as a whole and disregards variations in design, it appears that perioperative antibiotics significantly decrease the incidence of postoperative infection.

The question that remains therefore is not whether to use an agent for prophylaxis but rather which agent to use. Ledger et al.[24] published a proposed list of guidelines, which were subsequently revised and restated by

Johnson et al.,[20] on selecting a drug for perioperative administration: (1) The procedure must have a significant postoperative infectious morbidity or the patient must be thought to have an altered host response to infection. (2) The operative procedure must be associated with a high probability of microbial contamination. (3) The drug must be of low toxicity, must have an established record of patient safety, and must not be routinely used for serious infections. (4) The drug used must have a spectrum of activity that includes those microorganisms from the site of contamination that are most likely to cause infection. (5) The drug must achieve a reasonable tissue concentration during the procedure and be administered for a short duration. (6) The hospital must have a functioning infection surveillance program with established guidelines and maintain records of current antibiotic susceptibility patterns prior to initiating elective antibiotic prophylaxis for general use.

Although meant as guidelines, these tenets constitute a practical basis for establishing need for prophylaxis and provide the preliminary structure necessary to proceed in a given hospital or practice. Nevertheless, considering the overwhelming number of effective drugs available, the choice of antibiotic remains problematic.

The aminoglycosides, because of their potential ototoxicity and nephrotoxicity and because they require a second drug to provide adequate coverage, should not be considered for prophylaxis. Similarly, because of their potential maternal hepatotoxicity, the tetracyclines are not suited for this purpose. Of the remaining classes of antibiotics, the penicillins and cephalosporin-related antibiotics seem the best choices for prophylactic use in surgical delivery. These β-lactam antibiotics have been well studied in both gynecology and obstetrics and have been shown to effectively reduce the incidence of postoperative infection when administered prophylactically during the perioperative period. The previously stated guidelines can be helpful for selecting an agent since they suggest using a relatively inexpensive drug that is not used as therapy for serious infections. Although this appears simplistic, it must be understood that a drug chosen for prophylaxis should be one that is not used for nosocomial infections, including postoperative sepsis in a patient who received it for prophylaxis since resistance to it may have already developed.

For almost 20 years, we have recognized that, in general, any antibiotic given either prior to or early in the procedure (when umbilical blood flow has been terminated) will result in decreased maternal infection. Extrapolating from the most recent studies, it appears, moreover, that the duration of adequate drug levels in the tissues need not be prolonged. Therefore most drugs with a reasonable half-life (1 to 2 hours) should be as effective when given as a single dose as when given in multiple doses. Since numerous drugs with varying spectrums of antimicrobial activity have been studied, it is apparent that activity against all of the organisms present in the cervicovaginal flora is unnecessary. Although these organisms are frequently isolated from post–cesarean section infections, simply decreasing their concentration (rather than eradicating them) appears to be effective in reducing infection.

The appearance of resistance

It would seem that any antibiotic with a reasonable half-life and spectrum of activity that is both nontoxic and inexpensive would be an ideal candidate for prophylactic "drug of choice." However, just as we have become more sophisticated with respect to perinatology, we have also begun to understand the wider implications of our drug choices. Selective pressure by frequent use of antibiotics has already altered the effectiveness of some of our most well-accepted regimens. In many hospitals, for example, the majority of coliforms, for example, *Escherichia coli,* have become resistant to ampicillin. Although this type of selection pressure leading to bacterial resistance has been a concern of infectious disease specialists since antibiotic prophylaxis came into vogue, a direct cause and effect has been difficult to demonstrate. Nevertheless, Moellering and Wennersten,[25] using computer analysis, were able to show that an increase in the prevalence of infections due to gentamicin-resistant organisms coincided with increased use of the drug at the Massachusetts General Hospital.

Effects of antibiotics on host flora

The effect of an antibiotic on host flora has been evaluated by several investigators in patients undergoing cesarean section. Gibbs and Weinstein,[19] for example, isolated enterococci and *E. coli* from endometrial cultures up to 5 days after the procedure in subjects who received a combination of clindamycin plus gentamicin. In previously unpublished work, Ohm-Smith and Galask studied 38 women who had been scheduled to have an elective repeat cesarean section and who had been randomly assigned to receive either a cephalosporin or a placebo according to a double-blind protocol. Cultures were obtained in 34 of the 38 subjects both before operation and on the fourth or fifth postoperative day, 1 to 2 days after the study medication had been discontinued. Table I shows that, although the average number of aerobic and anaerobic organisms was similar in the two groups when the preoperative cultures were evaluated, an increased number of anaerobic organisms was seen in the postoperative placebo group. Fig. 1 shows that there was a marked increase in the percent of cultures positive for

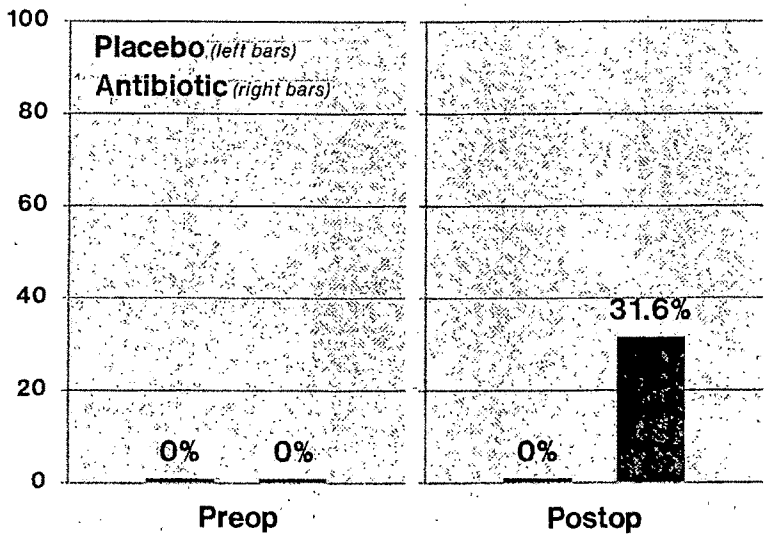


Fig. 1. Percent of cultures positive for cephalosporin-resistant gram-negative rods.

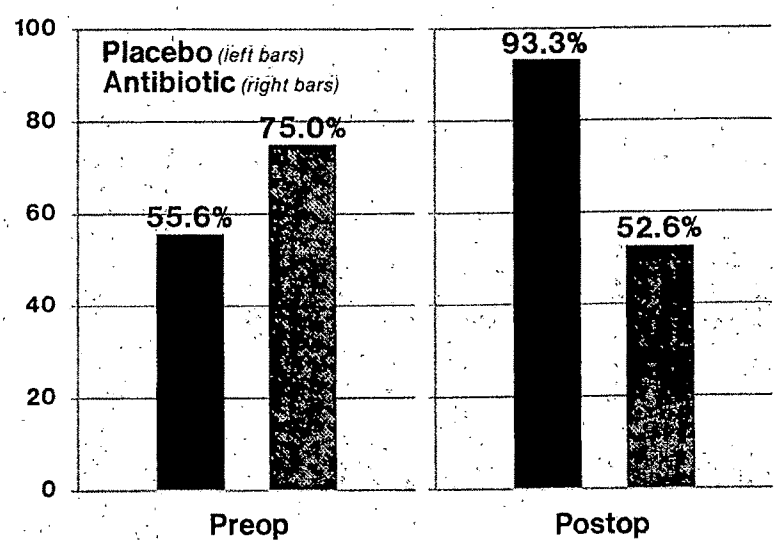


Fig. 2. Percent of cultures positive for anaerobic gram-negative rods.

Table I. Average number of different organisms per culture

Patient group and type of organism	*Preoperative cultures*	*Postoperative cultures*
Placebo		
Aerobic	2.5	3.1
Anaerobic	4.2	2.3
Antibiotic		
Aerobic	2.6	2.3
Anaerobic	4.5	2.7

cephalosporin-resistant gram-negative rods only in the treated group. Fig. 2 shows that there was an increase in percent of cultures positive for anaerobic gram-negative rods in the placebo group compared with a slight decrease in the treated group. Of greater clinical importance, however, were the marked increases in percent of cultures positive for both *Bacteroides asaccharolyticus* (Fig. 3) and *B. bivius* (Fig. 4) in the placebo group. *B. fragilis* and *B. distasonis* were also isolated with slightly greater frequency in the placebo group.

The results are disconcerting since they suggest that cervicovaginal flora can be affected, regardless of whether an antibiotic is used. Although no untoward problems with resistant organisms were noted in this or other studies, the effects of antibiotic selection remain apparent. Interestingly, a recent report by Ganesh et al.[18] suggests that trimethoprim-sulfamethoxazole lowered morbidity with a lesser effect on the flora. Further studies will be required to confirm this finding. An antibiotic that can decrease morbidity with minimal al-

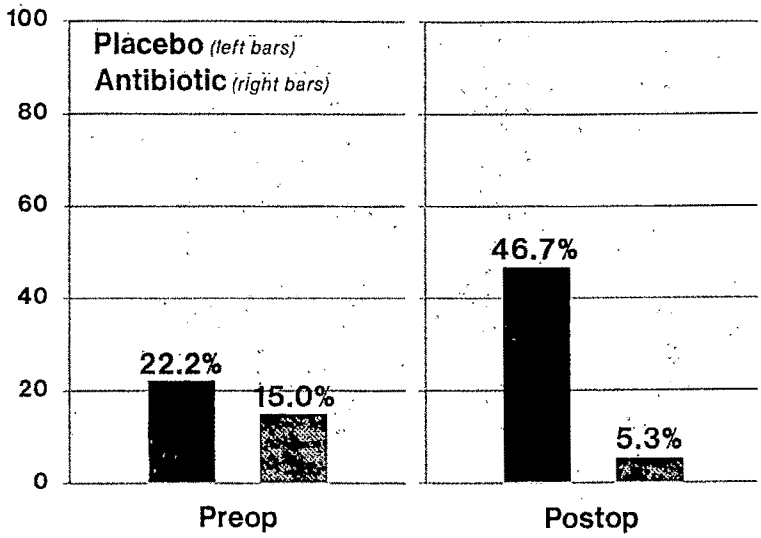


Fig. 3. Percent of cultures positive for *B. asaccharolyticus.*

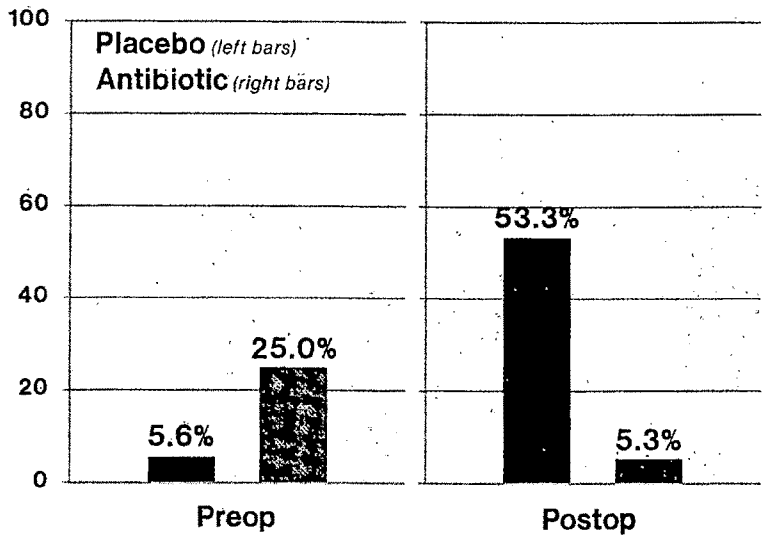


Fig. 4. Percent of cultures positive for *B. bivius.*

teration of the flora would be an excellent choice for prophylaxis, provided that the agent is nontoxic and inexpensive.

β-Lactamase induction

Concern about the development of microbial resistance resulting from indiscriminate use of perioperative antibiotics for prophylaxis has not previously been justified. Recent evidence suggests, however, that a certain type of bacterial resistance to the β-lactam antibiotics may be important when one is selecting drugs for prophylaxis. As generations of β-lactamase–resistant antibiotics were developed and released, it was believed that there would be a proportional rise in efficacy. Although improvements in our antibiotic armamentarium have succeeded in decreasing the use of combinations of two or three different drugs, disturbing reports in the literature have suggested that certain microorganisms rapidly developed resistance to these newer agents. Perhaps even more distressing is the possibility that current in vitro susceptibility testing may be inadequate to detect this type of antibiotic resistance when it occurs. Unlike resistance that could be explained by bacterial tolerance to the lytic effects of β-lactam antibiotics, which has been described in the past and recently reviewed,[26] it appears that a different type of resistance to the β-lactam antibiotics is occurring, the significance of which previously was not rec-

ognized. This resistance is due to the induction of a specific type of β-lactamase enzyme of the Richmond-Sykes type I group.[27] It has been suggested that these cephalosporinases are produced by the derepression of a control gene on the bacterial chromosome and not by a transferable plasmid.[28] Although resistance is not transferred to other strains, certain gram-negative enteric bacteria (mutant and wild-type organisms) can become resistant to the newer cephalosporins previously thought to be stable to the effects of β-lactamase enzymes. More interesting is the fact that with this type of resistance, the β-lactam ring does not undergo classic hydrolysis; rather, a stable complex is formed between the antibiotic and the enzyme.[29] The newer, relatively β-lactamase–stable cephalosporins that bind to the penicillin-binding proteins types I and III are affected the most. When present, the enzyme confers simultaneous resistance to most of the broad- and extended-spectrum penicillins, as well as to the second- and third-generation cephalosporins, including the cephamycins and the newer monobactams.

This Richmond-Sykes type I β-lactamase enzyme exerts its action within the periplasmic space of the induced organism. Antibiotics gain access to this space by transversing the outer membrane of the bacterium through protein channels called porins. Once inside the periplasmic space, an antibiotic must reach and bind to a specific type of protein-binding protein to affect cell wall synthesis. Classically, a β-lactamase produced by the cell binds to an antibiotic molecule, hydrolyzes the β-lactam ring, releases the inactive compound, and binds to another molecule. When sufficient enzyme-to-antibiotic concentrations are present, the organism is protected from the effects of the drug. It appears, however, that the resistance conferred by the Richmond-Sykes type I enzyme does not follow this classic pathway. When an organism has been induced (derepressed), it produces large quantities of enzyme, which bind to the newer, stable antibiotics and do not hydrolyze the molecules but remain bound to them, blocking their ability to attach to their specific penicillin-binding proteins. This blocking or trapping effect protects the organism and can develop during therapy for an infectious disease. Further, this type of resistance is generally undetected because of the low inoculum size (approximately 10^4), and thus insufficient number of organisms, used by commercial and hospital laboratories for antibiotic susceptibility testing. This is a clinically important point since it helps to explain antibiotic failure in a patient who is being treated with a reportedly effective drug.

We now recognize that certain of the newer β-lactam antibiotics can act as inducers of this class of β-lactamase enzymes. Clearly, if an antibiotic used perioperatively to prevent infection causes enzyme induction and the patient subsequently becomes infected, a number of the organisms present at the site would be resistant to most of the antibiotics logically used to treat the disease process. Thus it would be prudent not to use a drug for prophylaxis that has been shown to be associated with the production of inducible, nonhydrolytic enzymes. Apparently, the most potent inducers of this type of resistance are the newer β-lactamase–stable second- and third-generation cephalosporins. Cefoxitin, for example, has been reported to induce resistance to a number of cephalosporins as well as to the new, extended-spectrum penicillins.[30] It is fortunate that the organisms in which this resistance is most likely to occur—enteric bacteria such as *Enterobacter cloacae*[31]—are not frequently encountered in postoperative obstetric infections.

Comment

Clearly, advances have been made in our understanding and use of antibiotic prophylaxis in obstetrics. The efficacy of perioperative antibiotic administration in decreasing the incidence of serious infections has been demonstrated and accepted. We have been able to identify patients who are at high risk and benefit most from prophylaxis. The amount of drug administered to each patient has decreased. The practice of delaying antibiotic administration until the umbilical cord is clamped to protect the neonate has been widely accepted.

It is also apparent, however, that our understanding of the effects of perioperative antibiotic prophylaxis is still incomplete. Single-dose prophylaxis, which would further decrease the amount of drug received by the patient, has yet to be established as the accepted regimen. The identification of antibiotics or chemotherapeutics that would decrease morbidity while not markedly affecting the indigenous flora is only beginning. Certainly, the implications of inducible antibiotic resistance by certain drugs are vitally important and must be thoroughly evaluated. It seems that we have raised as many questions as we have answered and may still be as far from finding the ideal drug for prophylaxis as Ehrlich was in his search for a "Silver Bullet."

REFERENCES

1. Richards WR. An evaluation of the local use of sulfonamide drugs in certain gynecological operations. AM J OBSTET GYNECOL 1943;46:541.
2. Hesseltine HC, Thelen C. Sulfonamides as a prophylactic agent in conjunction with cesarean section. AM J OBSTET GYNECOL 1946;52:813.
3. Keettel WC, Plass ED. Prophylactic administration of penicillin to obstetric patients. JAMA 1950;142:324.
4. Burke JF. The effective period of preventive antibiotic action in experimental incisions and dermal lesions. Surgery 1961;50:161.
5. Bottoms SF, Rosen MG, Sokol RJ. The increase in the cesarean birth rate. N Engl J Med 1980;302:559.

6. Miller RD, Crichton D. Ampicillin prophylaxis in cesarean section. S Afr J Obstet Gynecol 1968;6:69.
7. Gibbs RS, Hunt JE, Schwarz RH. A follow-up study on prophylactic antibiotics in cesarean section. Am J Obstet Gynecol 1973;117:419.
8. Green SL, Sarubbi FA, Bishop EH. Prophylactic antibiotics in high-risk cesarean section. Obstet Gynecol 1978; 51:569.
9. Wong R, Gee CL, Ledger WJ. Prophylactic use of cefazolin in monitored obstetric patients undergoing cesarean section. Obstet Gynecol 1978;51:407.
10. Kreutner AK, DelBene VE, Delamar D, et al. Perioperative antibiotic prophylaxis in cesarean section. Obstet Gynecol 1978;52:279.
11. Weissberg SM, Edwards NL, O'Leary JA. Prophylactic antibiotics in cesarean section. Obstet Gynecol 1971;38: 290.
12. Moro M, Andrews M. Prophylactic antibiotics in cesarean section. Obstet Gynecol 1974;44:688.
13. Morrison JC, Coxwell WL, Kennedy BS, et al. The use of prophylactic antibiotics in patients undergoing cesarean section. Surg Gynecol Obstet 1973;136:425.
14. Gordon HR, Phelps D, Blanchard K. Prophylactic cesarean section antibiotics: maternal and neonatal morbidity before or after cord clamping. Obstet Gynecol 1979; 53:151.
15. Hawrylyshyn PA, Bernstein P, Papsin FR. Short-term antibiotic prophylaxis in high-risk patients following cesarean section. Am J Obstet Gynecol 1983;156:285.
16. Gonik B. Single- versus three-dose cefotaxime prophylaxis for cesarean section. Obstet Gynecol 1985;65:189.
17. Saltzman DH, Eron LJ, Kay HH, et al. Single-dose antibiotic prophylaxis in high-risk patients undergoing cesarean section. Obstet Gynecol 1985;65:655.
18. Ganesh V, Apuzzio JJ, Dispenziere B, et al. Single-dose trimethoprim-sulfamethoxazole prophylaxis for cesarean section. Am J Obstet Gynecol 1986;154:1113.
19. Gibbs RS, Weinstein AJ. Bacteriologic effects of prophylactic antibiotics in cesarean section. Am J Obstet Gynecol 1976;126:226.
20. Johnson SR, Ohm-Smith M, Galask RP. Prophylactic antibiotics in obstetrics and gynecology. In: Zuspan FP, Christian CD, eds. Controversy in obstetrics and gynecology. Philadelphia: WB Saunders, 1983, vol 3.
21. Phelan JP, Pruyn SC. Prophylactic antibiotics in cesarean section: a double-blind study of cefazolin. Am J Obstet Gynecol 1979;133:474.
22. Rothbard MJ, Mayer W, Wystepek A, et al. Prophylactic antibiotics in cesarean section. Obstet Gynecol 1975;45: 421.
23. Rehu M, Nilsson CG. Risk factors for febrile morbidity associated with cesarean section. Obstet Gynecol 1980; 56:269.
24. Ledger WJ, Gee C, Lewis WP. Guidelines for antibiotic prophylaxis in gynecology. Am J Obstet Gynecol 1975; 121:1038.
25. Moellering RC Jr, Wennersten C, Kunz LJ. Emergence of gentamicin-resistant bacteria: experience with tobramycin therapy of infections due to gentamycin-resistant organisms. J Infect Dis 1976;134(suppl):540.
26. Tomasz A. From penicillin-binding proteins to the lysis and death of bacteria: a 1979 view. Rev Infect Dis 1979;1:434.
27. Richmond MH, Sykes RB. The β-lactamases of gram-negative bacteria and their possible physiological role. In: Rose AH, Tempest DW, eds. Advances in microbial physiology. New York: Academic Press, 1973:31-85.
28. Gootz TD, Sanders CC, Goering RV. Resistance to cefamandole: depression of β-lactamases by cefoxitin and mutation in *Enterobacter cloacae*. J Infect Dis 1982;146:34.
29. Sanders CC. Novel resistance selected by the new expanded-spectrum cephalosporins: a concern. J Infect Dis 1983;147:585.
30. Sanders CC, Sanders WE Jr, Goering RV. In vitro antagonism of beta-lactam antibiotics by cefoxitin. Antimicrob Agents Chemother 1982;21:968.
31. Seeberg AH, Tolxdorff-Neutzling RM, Wiedemann B. Chromosomal β-lactamases of *Enterobacter cloacae* are responsible for resistance to third-generation cephalosporins. Antimicrob Agents Chemother 1983;23:918.

Single-dose prophylaxis for vaginal and abdominal hysterectomy

David L. Hemsell, M.D., Molly C. Heard, R.A., Brenda J. Nobles, Roger E. Bawdon, Ph.D., and Patricia G. Hemsell, R.A.
Dallas, Texas

The practice of administering perioperative antimicrobials to uninfected patients undergoing elective surgical procedures has been subjected to careful prospective investigative scrutiny during the past two decades. A wound classification system was developed, helping to identify procedures for which prophylaxis did not provide benefit to the patient. Guidelines for prophylaxis in hysterectomy were proposed and risk factors were sought. Pharmacokinetics and spectrum of antibacterial activity, although variables of paramount importance in predicting success in the treatment of established infections, were discovered to have questionable impact on prophylactic efficacy. Duration of antimicrobial administration has gradually diminished from the total length of hospital stay to a single preoperative dose without increased risk of infection; efficacy may be related to route of administration. The wisdom of using newer agents for prophylaxis is addressed and questioned. (Am J Obstet Gynecol 1987;157:498-501.)

Key words: Surgical prophylaxis, vaginal/abdominal hysterectomy

Antimicrobial administration to prevent infection after most elective surgical procedures, including hysterectomy, was popularized in the early 1950s. By the mid to late 1950s, it was a common practice to begin administering antimicrobials to patients scheduled for any surgical procedure from the time they were admitted to the hospital until they went home some 8 to 10 days later. Very few, if any, controlled studies delineating the benefits and risks of such a practice had been done at that time.

Specialists in internal medicine warned of the potential hazards of prolonged antimicrobial administration in noninfected persons, but their warnings went unheeded. Some patients undergoing primarily orthopedic and neurosurgical procedures developed superinfections as a result of such antimicrobial regimens. The serious sequelae and even deaths associated with these superinfections eventually led to radical changes in the approach to surgical prophylaxis.

Prospective studies were conducted in all surgical subspecialties to identify those surgical procedures for which the prophylactic administration of antimicrobials was beneficial. The first articles reporting the results of these studies began to appear in the gynecologic literature in the late 1960s[1] and have continued to appear since that time.

From the Department of Obstetrics and Gynecology, University of Texas Health Science Center at Dallas, Southwestern Medical School, Parkland Memorial Hospital.
Reprint requests: David L. Hemsell, M.D., Department of Obstetrics and Gynecology, University of Texas Health Science Center, Southwestern Medical School, Dallas, TX 75235.

Surgical classifications

The recent proliferation of newer antimicrobials has accentuated the importance of applying the 1975 guidelines of Ledger et al.[2] for surgical prophylaxis.

Hysterectomy was originally designated as a "clean" procedure because the postoperative infection rate, particularly of abdominal hysterectomy, was so low.[3] Hysterectomy has since appropriately been reclassified as a clean/contaminated surgical procedure because it is performed in an area containing significant potential pathogens as part of the normal flora. Host defense mechanisms are paramount in the prevention of infection after elective hysterectomy. Since host defense mechanisms are impaired in debilitated and/or immunodeficient patients, these women are at increased risk for infection after hysterectomy or after any other surgical procedure. Low socioeconomic status is another, relatively universal risk factor for increased postoperative infection. Even with an intact immune system, an operative site grossly contaminated with a substantial inoculum will also usually result in postoperative infection.

Applying the principles of good surgical technique is also important in decreasing the incidence of postoperative infection. In addition to asepsis in the operating room, which is standard practice, many surgeons advocate douching the evening before and the morning of hysterectomy. Since serum and blood provide an ideal medium for the growth of bacteria that compose the normal flora of the lower reproductive tract (which are inoculated into the operative site), adequate hemostasis and closing of dead space are imperative. Creating small pedicles and gentle handling

of tissues diminish the volume of necrotic tissue that might also provide a nidus for subsequent infection.

Bacterial isolates

A total of 2298 gram-positive and gram-negative aerobic and anaerobic bacteria have been isolated from endocervical cultures obtained from 644 women before vaginal or abdominal hysterectomy in our hospital during the past several years (unpublished data).[4] Fifty-eight percent of the isolates were aerobic and 42% were anaerobic bacteria. Gram-positive cocci accounted for 67% of aerobic bacteria isolated; of these, 33% were *Streptococcus faecalis* (enterococci), 33% were *Staphylococcus epidermidis*, and 16% were group B streptococci. *Escherichia coli* was the predominant gram-negative isolate and accounted for 21% of the aerobic bacteria isolated. Nine percent of gram-negative aerobic isolates were *Enterobacter* sp., *Proteus* sp., and *Klebsiella* sp. Forty-three percent of the anaerobic bacteria were gram-positive cocci and 26% were *Bacteroides bivius*. An additional 14% were members of other *Bacteroides* sp. The *Bacteroides fragilis* group accounted for 12% of preoperative anaerobes isolated. Pathogens responsible for postoperative pelvic infection are derived from these bacteria.

Incidence and type of infection

Irrespective of surgical approach, infection developing in the pelvis after hysterectomy is potentially prevented by the prophylactic administration of an antimicrobial. Neither cystitis nor pyelonephritis is prevented by prophylaxis, and elective operations should not be scheduled or performed if urinary pathogens are present. Similarly, neither microatelectasis nor pneumonia is prevented by perioperative prophylaxis, and no respiratory tract inflammation should be present when elective operation is performed. Many placebo-controlled prospective studies, as well as our own experience, have indicated that prophylactic antibiotics do not diminish the occurrence of asymptomatic temperature elevation not associated with infection. Finally, since the incidence of abdominal incision infection is so low, prophylaxis does not significantly alter its occurrence.

Although the level of infection necessary to justify perioperative antimicrobial prophylaxis for hysterectomy is a matter of considerable debate, many have indicated an incidence of 10% as a breakpoint; surgeons' goals include no postoperative infections. This may result not only in prolonged administration of perioperative antimicrobials but also in antimicrobial administration for temperature elevation after hysterectomy, whether other evidence of infection is or is not present at the time. The latter practice does not reflect failure of the regimen given for prophylaxis or elevated infection rates as much as the anxiety level of the surgeon. The practice does, however, make it difficult to accurately measure infection rates.

Risk factors

It is difficult to identify uniform risk factors for infection after hysterectomy other than those previously mentioned. Just as the incidence of infection after hysterectomy varies from hospital to hospital, so may the risk factors. While it has been reported that phase of the menstrual cycle is related to an altered incidence of infection after hysterectomy, because of the altered microbiologic features of the lower reproductive tract during different phases of the cycle,[5] the majority of studies (including our experience) indicate that there is no increased risk during either phase of the menstrual cycle.[6, 7] Further, neither preoperative endocervical bacteria nor intraoperative cardinal ligament or other surgical site bacteria that are recovered are predictive of postoperative infection.[8-11] The duration of the surgical procedure has been reported as a risk factor by some investigators[12] but has not been confirmed in more than 2000 women evaluated prospectively in our hospital. Training and experience of the surgeon also appear unrelated to risk of postoperative infection. Interestingly, results of many comparative clinical trials indicate that the pharmacokinetics and antimicrobial spectrum of an agent selected for prophylaxis, although of paramount importance in determining therapeutic efficacy for established infection, seem to have little impact on prophylactic efficacy.

Despite almost universal acceptance of the fact that women undergoing vaginal hysterectomy require antimicrobial prophylaxis, there are many private practitioners who do not routinely administer prophylactic antibiotics because of low infection rates. There are several categories of women who appear to be at higher risk for development of infection after vaginal hysterectomy. Age is a factor, with both younger[12] and older[13] women presenting an increased risk. Diabetes is also a risk factor.[13] Additional procedures such as colporrhaphy or adnexectomy may increase the risk for postoperative infection,[10, 14] as does postoperative anemia in our hospital. The postoperative course for women who undergo vaginal hysterectomy, however, is much more benign and shorter than that for women undergoing an abdominal hysterectomy.

The abdominal approach increases the risk for infection after hysterectomy.[12, 13, 15] Older age,[13, 14] obesity,[16] and diabetes[13] may be risk factors that increase infection after abdominal hysterectomy. Obesity appears to pose a problem primarily in healing of the abdominal incision, and mechanical management of the incision appears to be more important that the administration of antimicrobial prophylaxis. Excessive cau-

tery increases the incidence of nonunion of the abdominal incision.[17] Preceding procedures such as dilation and curettage or conization more than 72 hours but less than 4 weeks before hysterectomy appear to increase the risk of postoperative infection after abdominal hysterectomy[18] as well as vaginal hysterectomy.[19] Since there is significant controversy over the requirement for perioperative prophylaxis at abdominal hysterectomy,[20] individual determination is required. The use of an intraperitoneal wash has not been as well studied in the context of abdominal hysterectomy as it has been with cesarean section, but preliminary data indicate than an intraoperative wash may significantly reduce postoperative infection.

Single-dose prophylaxis

With increased experience, the duration of prophylactic antimicrobial administration has decreased from 7 days, to 5 days, to 3 days, to 1 day, to three perioperative doses by the mid-1980s. Most studies involved comparisons of multiple doses, although a few investigators reported results with single doses of various antimicrobials. Recently, attention to the costs of health care have rekindled interest in single-dose prophylaxis. A single dose of an antimicrobial for prophylaxis apparently can be effective in reducing pelvic infection after hysterectomy. The advantages of single-dose prophylaxis are numerous and obvious. In general, it is less expensive to administer one dose rather than multiple doses of an antibiotic, depending, in part, on cost of drug per gram. When less antibiotic is given, less nursing time and less pharmacy time are required. Moreover, use of space in both pharmacy and nursing units is improved. Prospective comparative studies showed that recovery of organisms resistant to an antimicrobial regimen is significantly higher after three doses than after one dose.[21, 22] Such results indicate that perhaps as few as three doses may significantly alter host flora and contribute to the development of selective resistance in bacteria. Clearly, the fewer doses administered, the less likely it is that the patient will develop either toxic or allergic adverse reactions. Having identified a need for prophylaxis and accepting the fact that a single dose is all that is required, two problems remain: which agent to administer and how to administer it.

Routes of administration

If the antibiotic is administered intramuscularly, serum and tissue levels will generally be lower but will persist longer than with intravenous administration. According to data from an animal skin model,[23] the window for efficacy of perioperative prophylaxis is approximately 3 hours in duration and is maximal if administration occurs relatively soon before operation. Although our initial impression was that patient benefits would be greater in a teaching institution if the agent were administered intramuscularly because of prolonged concentrations, more recent experience indicates that infection rates may be lower if the agent is given intravenously during 15 to 30 minutes in the operating room after the intravenous line is in place but prior to anesthesia. With the patient awake, it is possible to identify adverse reactions before the entire dose is given.

Single-dose agents

The first-generation cephalosporins cefazolin,[24] cephalothin,[25] and cephradine[26] have been evaluated as single agents in vaginal hysterectomy prophylaxis and have proved effective; however, they have not been evaluated in women undergoing abdominal hysterectomy. Single-dose cefoxitin,[21, 22] cefonicid,[27] cefotetan,[28] ceforanide,[29] cefotaxime,[30] and ceftriaxone[13] are second- and third-generation cephalosporins that have established clinical efficacy in both vaginal and abdominal hysterectomy prophylaxis. Single-dose piperacillin (unpublished data) was recently investigated in both procedures in our hospital and was found to be as effective as triple-dose cefoxitin in preventing postoperative pelvic infection.

Cefonicid, ceforanide, cefotaxime, and cefotetan are currently approved by the Food and Drug Administration for single-dose prophylaxis in hysterectomy. Prophylactic efficacy appears equivalent, although significant differences in serum and tissue half-life and cost per gram exist. Finally, we need to consider whether to administer second- and third-generation cephalosporins or newer penicillins for prophylaxis and thus possibly jeopardize their future efficacy as therapeutic agents when a first-generation cephalosporin may be as efficacious and less expensive. If efficacy is indeed equivalent, it would seem wise to continue with the first-generation agents.

REFERENCES

1. Goosenberg J, Emich JP Jr, Schwarz RH. Prophylactic antibiotics in vaginal hysterectomy. Am J Obstet Gynecol 1969;105:503-6.
2. Ledger WJ, Gee C, Lewis WP. Guidelines for antibiotic prophylaxis in gynecology. Am J Obstet Gynecol 1975; 121:1038-45.
3. Ad Hoc Committee of the Committee on Trauma, Division of Medical Sciences, National Academy of Sciences, National Research Council. Postoperative wound infections: the influence of ultraviolet irradiation of the operating room and of various other factors. Ann Surg 1964;160(suppl):1-84.
4. Heard ML, Bawdon RE, Hemsell DL, Nobles BJ. Susceptibility profiles of potential aerobic and anaerobic pathogens isolated from hysterectomy patients. Am J Obstet Gynecol 1984;149:133-43.
5. Thadepalli H, Savage EW Jr, Salem FA, Roy I, Davidson

EC Jr. Cyclic changes in cervical microflora and their effect on infections following hysterectomy. Gynecol Obstet Invest 1982;14:176-83.
6. Tashjian JH, Coulam CB, Washington JA II. Vaginal flora in asymptomatic women. Mayo Clin Proc 1976;51: 557-61.
7. Levison ME, Corman LC, Carrington ER, Kaye D. Quantitative microflora of the vagina. AM J OBSTET GYNECOL 1977;127:80-5.
8. George JW, Ansbacher R, Otterson WN, Rabey F. Prospective bacteriologic study of women undergoing hysterectomy. Obstet Gynecol 1975;45:60-3.
9. Grossman JH III, Adams RL, Hierholzer WJ Jr, Andriole VT. Endometrial and vaginal cuff bacteria recovered at elective hysterectomy during a trial of antibiotic prophylaxis. AM J OBSTET GYNECOL 1978;130:312-6.
10. Hemsell D, Hemsell P, Nobles B, Heard M, Bawdon R. Moxalactam versus cefazolin prophylaxis for vaginal hysterectomy. AM J OBSTET GYNECOL 1983;147:379-85.
11. Hemsell DL, Johnson ER, Bawdon RE, Hemsell PG, Heard ML, Nobles BJ. Cefoperazone and cefoxitin prophylaxis for abdominal hysterectomy. Obstet Gynecol 1984;63:467-72.
12. Shapiro M, Munoz A, Tager IB, Schoenbaum SC, Polk BF. Risk factors for infection at the operative site after abdominal or vaginal hysterectomy. N Engl J Med 1982;307:1661-6.
13. Hemsell DL, Menon MO, Friedman AJ. Ceftriaxone or cefazolin for prophylaxis for the prevention of infection after vaginal hysterectomy. Am J Surg 1984;148:22-6.
14. Gallup DG. Modifications of celiotomy techniques to decrease morbidity in obese gynecologic patients. AM J OBSTET GYNECOL 1984;150:171-8.
15. Dicker RC, Greenspan JR, Strauss LT, et al. Complications of abdominal and vaginal hysterectomy among women of reproductive age in the United States, The Collaborative Review of Sterilization. AM J OBSTET GYNECOL 1982; 144:841-8.
16. Pitkin RM. Abdominal hysterectomy in obese women. Surg Gynecol Obstet 1976;142:532-6.
17. Ledger WJ. Prevention, diagnosis, and treatment of postoperative infections. Obstet Gynecol 1980;55(suppl 5): 203S-6S.
18. Laubach JB, McGanity WJ. Hysterectomy post conization of the cervix. AM J OBSTET GYNECOL 1965;91:437-42.
19. van Nagell JR, Roddick JW, Cooper RM, Triplett HB. Vaginal hysterectomy following conization in the treatment of carcinoma in situ of the cervix. AM J OBSTET GYNECOL 1972;113:948-51.
20. Duff P. Antibiotic prophylaxis for abdominal hysterectomy. Obstet Gynecol 1982;60:25-9.
21. Hemsell DL, Heard ML, Nobles BJ, Hemsell PG. Single-dose cefoxitin prophylaxis for premenopausal women undergoing vaginal hysterectomy. Obstet Gynecol 1984;63: 285-90.
22. Hemsell DL, Reisch J, Nobles B, Hemsell PG. Prevention of major infection after elective abdominal hysterectomy: individual determination required. AM J OBSTET GYNECOL 1983;147:520-8.
23. Burke JF. The effective period of preventive antibiotic action in experimental incisions and dermal lesions. Surgery 1961;50:161-8.
24. Lett WJ, Ansbacher R, Davison BL, Otterson WN. Prophylactic antibiotics for women undergoing vaginal hysterectomy. J Reprod Med 1977;19:51-4.
25. Hamod KA, Spence MR, Rosenshein NB, Dillon MB. Single-dose and multidose prophylaxis in vaginal hysterectomy: a comparison of sodium cephalothin and metronidazole. AM J OBSTET GYNECOL1980;136:976-9.
26. Mendelson J, Portnoy J, De Saint Victor JR, Gelfand MM. Effect of single and multidose cephradine prophylaxis on infectious morbidity of vaginal hysterectomy. Obstet Gynecol 1979;53:31-5.
27. Maki DG, Lammers JL, Aughey DR. Comparative studies of multi-dose cefoxitin vs. single-dose cefonicid for surgical prophylaxis in patients undergoing biliary tract operations or hysterectomy. Rev Infect Dis 1984;6(suppl): S887-95.
28. Orr JW Jr, Varner RE, Kilgore LC, Holloway RC, McDiarmid M. Cefotetan versus cefoxitin as prophylaxis in hysterectomy. AM J OBSTET GYNECOL 1986;154:960-3.
29. Bristol Laboratories. Precef. Package Circular, July 1984.
30. Roy S, Wilkins J. Single-dose cefotaxime versus three to five dose cefoxitin for prophylaxis of vaginal or abdominal hysterectomy. J Antimicrob Chemother 1984; 14(suppl B):217-21.

Single-dose versus multiple-dose piperacillin prophylaxis in primary cesarean operation

Stanley A. Gall, M.D., and Gale B. Hill, Ph.D.
Chicago, Illinois, and Durham, North Carolina

A prospective, randomized double-blind study was undertaken to determine the safety and efficacy of single-dose versus multiple-dose piperacillin for primary cesarean operation prophylaxis. After informed consent was obtained, patients received either 4 gm of piperacillin intravenously as a single dose or three 2 gm doses of piperacillin intravenously 4 hours apart. One hundred sixteen patients were evaluated for response; postoperative infection at the operative site was prevented in 52/60 (86.6%) who received the single dose and 53/56 (94.6%) who received multiple doses. The types of postoperative infections that occurred were similar in those patients in each group who developed infection and all responded well to therapeutic drugs. No adverse reactions to piperacillin were noted. Demographic characteristics and risk factors for infection were comparable for the two groups. These data support the efficacy of single-dose prophylaxis with an appropriate antibiotic agent. (AM J OBSTET GYNECOL 1987;157:502-6.)

Key words: Single-dose/multiple-dose prophylaxis, postoperative infection

The concept of administering perioperative antibiotics as prophylaxis for cesarean operation is widely accepted.[1-3] The basic science concepts and early principles of such prophylaxis were first clearly described by Burke.[4] In the last 20 years, many investigators have studied different antibiotics, doses, and durations of therapy. In addition, factors associated with an increased risk of clinical infection have been described. Results of many studies suggest that perioperative antibiotics reduce the incidence of postoperative infection, that many antibiotics seem to offer similar benefit, that maximum doses should be used, and that the duration of therapy should be no longer than 24 hours after operation. More recent data suggest that a perioperative course of antibiotics of less than 24 hours may be equally effective,[5-7] but not all investigators agree.[8] This study was designed to compare the efficacy and safety of a single dose of piperacillin with those of a three-dose regimen in preventing postoperative infection in women undergoing primary cesarean section.

Study design

The investigation was a double-blind, randomized comparison of two prophylactic dosage regimens of piperacillin. One treatment group received a single 4 gm dose of piperacillin intravenously and the other, a total of three 2 gm doses of the antibiotic given 4 and 8 hours after the first dose.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Illinois School of Medicine, and the Department of Obstetrics and Gynecology, Duke University Medical Center.
Reprint requests: Stanley A. Gall, M.D., Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Illinois School of Medicine, Chicago, IL 60680.

Patients admitted to Duke University Hospital who were undergoing primary cesarean section and who were considered to be at increased risk for postoperative infection were eligible for inclusion. Increased risk was determined according to guidelines described by Gibbs.[9] The presence of any of the following constituted increased risk: labor or ruptured membranes for ≥6 hours, internal fetal monitoring >9 hours, use of an intrauterine pressure catheter, or three or more vaginal examinations.

Patients who met the entrance requirements and gave informed consent underwent a complete medical history, physical examination, and laboratory tests of a complete blood count with platelet count, blood urea nitrogen, alkaline phosphatase, serum glutamic oxaloacetic transaminase, and urinalysis. Urine cultures were obtained prior to operation if the patient's history suggested previous urinary tract infections.

On entering the study, patients were assigned to one of two treatment groups according to a computer-generated randomization schedule. Patients assigned to receive a single dose of piperacillin received 4 gm intravenously immediately after umbilical cord clamping. These patients then received intravenous infusions of normal saline solution 4 and 8 hours after the initial single dose of piperacillin. Patients in the multiple-dose group received 2 gm of piperacillin intravenously immediately after umbilical cord clamping. Additional doses of 2 gm were given intravenously at 4 and 8 hours after the initial dose. Each patient was monitored daily for adverse clinical reactions and local tolerance.

Clinical findings used to determine the presence of infection were as follows: (1) Operative site infection with a presumptive diagnosis based on (a) a temperature of ≥39° C in the first 24 hours after operation or

Table I. Demographic characteristics, risk factors, and indications for cesarean section

Patient characteristics	*Single dose (N = 60)*	*Multiple dose (N = 56)*	*p Value*
Age (yr) (mean and range)	23.2 (15-43)	23.3 (130-40)	NS
Weight (pounds) (mean and range)	164 (92-268)	173 (100-383)	NS
Height (inches) (mean and range)	63.3 (58-69)	62.9 (56-69)	NS
Race (n)			
Black	40	42	
White	17	11	
Other	3	3	
Gravidity	1.9	2.0	NS
Gestational age (wk)	37.2 (24-42)	37.6 (25-42)	NS
Ruptured membranes (n)	38/60	41/56	NS
Duration of ruptured membranes (n)	12.8	10.6	NS
Labor (n and %)	51/60 (85%)	45/56 (80%)	NS
Duration of labor (hr) (mean)	15.7	14.6	NS
Internal fetal monitoring (n)	32/60	31/56	NS
Duration of monitoring (hr) (mean)	6.6	6.4	NS
Intrauterine pressure catheter (n and %)	12/60 (20%)	11/56 (20%)	NS
Vaginal examinations (n) (mean)	5.6	5.8	NS
Total No. of risk factors			
None	6	4	NS
1	10	9	
2	19	21	
3	10	10	
4	15	12	

(b) a temperature of ≥38.5° C on two consecutive measurements at least 4 hours apart, 24 hours after delivery, and the presence of two of the following—uterine tenderness, foul-smelling cervical discharge, and no evidence that fever was associated with another cause; (2) wound infection, indicated by the presence of local induration and tenderness with purulent drainage from the incision; (3) urinary tract infection, with a urine culture containing $\geq 10^5$ organisms per milliliter; (4) lower respiratory tract infection, with radiologic evidence of pulmonary infiltrate(s) and fever.

If the patient manifested signs of postoperative infection, the following cultures were obtained: for endometritis, endometrial culture and/or culdocentesis; for wound infection, wound exudate; for urinary tract infection, urine; and for all patients evaluated for postoperative infection, blood cultures. Specimens taken for microbiologic culture were processed as previously described.[10, 11]

Results

A total of 129 patients were accessed to the study, with 64 patients in the single-dose group and 65 in the multiple-dose group. Thirteen patients, four in the single-dose group and nine in the multiple-dose group, could not be evaluated because of errors in antibiotic administration. In the single-dose group, two patients could not be evaluated because they had received other antibiotics within the previous 7 days, one was receiving concomitant antibiotics, and the fourth patient was given the prophylactic antibiotic too late. In the multiple-dose group, six of nine patients who could not be evaluated had received antibiotics within the preceding 7 days, and one each had received concomitant antibiotics, had received antibiotics too late, and had not received the first dose. The following analyses were based on the 116 remaining patients.

Table II. Indications for cesarean section

	n	%
Fetal distress	38/129	29.5
Cephalopelvic disproportion	37/129	28.7
Failure to progress	37/129	28.7
Preeclampsia	20/129	15.5
Breech	20/129	15.5
Two or more indications	87/129	67.4

Demographic characteristics and risk factors for infection are listed in Table I for the single-dose (60 patients) and multiple-dose (56 patients) antibiotic groups. There were no significant differences noted for any of these characteristics, and the two treatment groups were considered comparable. The 13 patients who could not be evaluated were demographically similar to the 116 patients who could be evaluated. The mean numbers of risk factors for infection were 2.3 ± 0.2 in the single-dose group and 2.4 ± 0.2 in the multiple-dose group.

Fetal distress, cephalopelvic disproportion, and failure to progress each accounted for about one third of the indications for operation. Most patients (87 of 129, 67.4%) had two or more indications for cesarean section (Table II).

Durations of operation were 53.9 minutes (range, 29 to 87) in the single-dose group and 55.0 minutes (range,

Table III. Bacteria isolated from operative site infection

*Patient and drug regimen**	*Specimen source*	*Culture results*
013 (SD)	Endometrial	Group B *Streptococcus* *Corynebacterium* sp. *Bacteroides bivius* *Lactobacillus fermentans* (anaerobic) *Peptostreptococcus tetradius* *Mycoplasma hominis*
036 (SD)	Culdocentesis	No growth
039 (SD)	Endometrial	Group B *Streptococcus* *Corynebacterium* sp. *Klebsiella pneumoniae* Coagulase-negative *Staphylococcus* Viridans streptococci, 2 types *B. bivius* *Peptostreptococcus anaerobius* *Ureaplasma urealyticum*
043 (SD)	Endometrial tissue	*K. pneumoniae* Enterococcus *Escherichia coli* *U. urealyticum*
059 (SD)	Endometrial	*K. pneumoniae* Viridans *Streptococcus* *Corynebacterium* sp., 2 types Coagulase-negative *Staphylococcus* *M. hominis*
063 (SD)	Not cultured	
127 (SD)	Endometrial	*Corynebacterium* sp. Coagulase-negative *Staphylococcus* Viridans *Streptococcus* *Bacteroides* sp. *B. bivius* *P. anaerobius* *P. tetradius* *Lactobacillus* sp. (anaerobic)
130 (SD)	Culdocentesis	*Gardnerella vaginalis* *Corynebacterium* sp.
	Blood	*Streptococcus morbillorum*
014 (MD)	Endometrial	Viridans *Streptococcus* *Lactobacillus* sp. *B. bivius* *Lactobacillus* sp. (anaerobic)
074 (MD)	Endometrial	Enterococcus *E. coli* Coagulase-negative *Staphylococcus* *Corynebacterium* sp. *Proteus* sp. *Bacteroides* sp. *B. fragilis* group
080 (MD)	Endometrial	Group B *Streptococcus* Viridans *Streptococcus* *B. bivius* *Bacteroides* sp.

*SD = Single-dose regimen prophylaxis; MD = Multiple-dose regimen prophylaxis.

25 to 120) in the multiple-dose group. Patients received their first intraoperative doses at a mean of 3.7 minutes (range, −11 to 17) in the single-dose group and at an average of 3.2 minutes (range, −17 to 22) in the multiple-dose group.

Response to prophylaxis was deemed satisfactory if no infection ensued at the operative site. In the 116 patients who could be evaluated, a satisfactory response was seen in 52 of 60 (86.6%) of the single-dose group and 53 of 56 (94.6%) of the multiple-dose group (p = NS; Fisher's exact test). Operative site infections occurred in eight of 60 (13.4%) patients who received single-dose antibiotics, including seven patients with endometritis and one patient with endometritis and septicemia. Three of 56 (5.4%) patients in the multiple-dose group developed operative site infections, including two patients with endometritis and one patient with endometritis and wound infection. Culture results for the 11 patients with failure of antibiotic prophylaxis are given in Table III. Susceptibility to piperacillin was de-

termined for these isolates; only one strain of *Escherichia coli* was reported to be resistant (minimum inhibitory concentration >128 μg/ml).

Six patients judged to have satisfactory prophylactic courses developed "non–operative site" infections after leaving the hospital but within the 6-week follow-up period. These included one patient in the single-dose group who developed pelvic inflammatory disease at 6 weeks post partum and five patients in the multiple-dose group (four developed urinary tract infections and one developed an upper respiratory infection). Since these infections all occurred at least several weeks after the patient was discharged from the hospital, they were not believed to be related to operation.

Postoperative antibiotics were administered in the hospital to each patient having an unsatisfactory response to prophylaxis. In-hospital patients received primarily cefamandole, 2 gm every 6 hours, and clindamycin, 600 mg every 6 hours (seven patients), or cefamandole and metronidazole, 500 mg every 6 hours (one patient). One patient each was treated with cefamandole alone, moxalactam, 2 gm every 8 hours, and moxalactam plus clindamycin. All patients responded satisfactorily.

No adverse reactions to piperacillin were noted in either of the two treatment groups. Hematologic parameters were not affected and both dosage regimens were well tolerated.

Comment

The recent literature[2, 3] reports that patients undergoing primary cesarean section have a 10% to 85% risk of postoperative infection, depending on factors such as socioeconomic status and the presence of increased risk factors. The incidence of infection in the patient at increased risk is 40% to 85%. In contrast, the risk of morbidity in patients undergoing elective repeat cesarean operation is less than 20%. A previous study performed at the same hospital demonstrated infection rates of 40.8% in placebo-treated women undergoing primary cesarean section and 22% in those undergoing elective repeat cesarean operations when prophylactic antibiotics were not administered.[3]

In this investigation, the rate of postoperative infection in patients given a single 4 gm dose of prophylactic piperacillin was not statistically different from that observed for patients given a three-dose regimen (total, 6 gm). Similar efficacy with single-dose prophylaxis has been described previously for other antibiotics. However, because of the relatively small number of patients in each group, it is not possible to exclude a beta error. Importantly, there was no difference in the severity of the infections that developed in patients from either group, and no patient developed either septic thrombophlebitis or a pelvic abscess requiring surgical drainage. In previous studies comparing cefazolin with other antibiotics, no statistical difference was observed with respect to efficacy, although the cefazolin-treated patients seemed to be more seriously ill when they became clinically infected.

Group B streptococci were isolated from the infected endometria of two patients in the single-dose group and one patient in the multiple-dose group. In a previous study[3] with a 24-hour perioperative course of cefazolin for cesarean section prophylaxis, no group B streptococci were isolated from patients with postoperative infections. Although these observations are limited and may therefore be spurious, one wonders whether the shorter dosing schedules eliminate group B streptococci as efficiently as protocols that utilize a 24-hour postoperative course. This may be an important consideration as an increasing number of protocols dictate shorter prophylactic courses.

In this investigation, the prophylactic efficacy of a single 4 gm dose of piperacillin was not statistically different from 2 gm doses given 4 hours apart. An advantage of this study in comparing single-dose versus multiple-dose regimens was the use of the same drug in both treatment groups. This avoids the problem of confounding variables related to different spectra of activity and pharmacokinetics when two different drugs are compared for different dosing regimens.[6, 7] Results obtained with either dosing regimen of piperacillin were acceptable and comparable with those reported in other recently published studies. Some caution is appropriate, however, regarding the conclusion that single-dose and multiple-dose regimens are truly equivalent. Because of the small numbers of patients tested, the power of this trial (beta error) may be too great.

Nevertheless, if some differences in efficacy between the two regimens do exist, they apparently are not substantial. We conclude therefore that the results of this study support the use of single-dose prophylaxis with an appropriate agent for its convenience.

REFERENCES

1. Miller RD, Crichton D. Ampicillin prophylaxis in cesarean section. S Afr J Obstet Gynecol 1968;6:69.
2. Gibbs RS, DeCherney AH, Schwarz RH. Prophylactic antibiotics in cesarean section: a double-blind study. Am J Obstet Gynecol 1972;114:1048.
3. Gall SA. The efficacy of prophylactic antibiotics in cesarean section. Am J Obstet Gynecol 1979;134:506.
4. Burke JF. Pre-operative antibiotics. Surg Clin North Am 1963;43:665.
5. Saltzman DH, Eron LJ, Kay HH, Sitos JG. Single-dose antibiotic prophylaxis in high-risk patients undergoing cesarean section. Obstet Gynecol 1985;65:655.
6. MacGregor JA, French JI, Makowski E. Single-dose cefotetan versus multidose cefoxitin for prophylaxis in cesarean section in high-risk patients. Am J Obstet Gynecol 1986;154:955.
7. Varner MW, Werner CP, Petzold BS, Galask RP. Com-

parison of cefotetan and cefoxitin as prophylaxis in cesarean section. AM J OBSTET GYNECOL 1986;154:951.
8. Elliott JP, Freeman RK, Dorchester W. Short versus long course of prophylactic antibiotics in cesarean section. AM J OBSTET GYNECOL 1982;143:740.
9. Gibbs RS. Clinical risk factors for puerperal infection. Obstet Gynecol 1980;55:178S.
10. Gall SA, Addison WA, Hill GB. Moxalactam therapy for obstetrical and gynecological infections. Rev Infect Dis 1982;4(suppl):S701.
11. Gall SA, Kohan AP, Ayers OM, Hughes GE, Addison WA, Hill GB. Intravenous metronidazole or clindamycin with tobramycin for therapy of pelvic infections. Obstet Gynecol 1981;57:51.

Cost-effective use of antibiotic prophylaxis for cesarean section

Larry C. Ford, M.D., Hunter A. Hammil, M.D., and Thomas B. Lebherz, M.D.
Los Angeles, California

Clinical experience has indicated that the prophylactic use of antibiotics reduces infectious morbidity in patients undergoing cesarean section. Several factors must be considered (for instance, in vivo and in vitro efficacy, patient allergies, side effect profiles, status of host defenses, the total cost of therapy, and the risk of selecting resistant organisms that cause superinfections) before prescribing prophylactic antibiotic drugs for this indication. Moreover, medical-legal consequences associated with potential postpartum infections must be considered in assessing the costs and impact of a prophylactic regimen. Results of comparative antibiotic trials in indigent patients undergoing cesarean section demonstrated differing rates of successful antibiotic prophylaxis: piperacillin, 98%; cefoxitin, 91%; cephalothin and ceftazidime, 82%; cefotaxime, 80%; and ampicillin, 77%. Although the acquisition costs of antibiotics vary greatly, these costs are dwarfed by the substantial cost savings that can be realized by use of broad-spectrum antibiotics, which, in our hands, have resulted in reduced laboratory and pharmacy expenses and decreased hospital stays for both mother and neonate. Controlled studies designed to investigate microbiologic as well as clinical efficacy of antibiotics are indicated. Further refinements in individualizing antibiotic regimens according to patient population should be sought. (AM J OBSTET GYNECOL 1987;157:506-10.)

Key words: Cesarean section, prophylaxis, chemiluminescence, chemotaxis

Antibiotic prophylaxis of infection for women undergoing cesarean section differs from that used in gynecologic operations and other surgical procedures because of the presence of the fetus. In contradistinction to other surgical procedures, in which it is desirable to have effective antimicrobial levels present in the tissues at the time of operation,[1] antibiotics are usually withheld from women undergoing cesarean section until after the cord is clamped. Despite this difference in timing of antibiotic administration, however, clinical experience has shown that the incidence of postpartum infection can be decreased by the use of antibiotics.[2-8]

From the Department of Obstetrics and Gynecology and Jonsson Comprehensive Cancer Center, University of California at Los Angeles Medical Center.
Reprint requests: Larry C. Ford, M.D., Department of Obstetrics and Gynecology, University of California at Los Angeles Medical Center, Los Angeles, CA 90024-1740.

Antimicrobial selection

Identifying pathogens. Several factors should be evaluated when one is selecting an antimicrobial regimen for cesarean section prophylaxis. For example, the presence of potentially pathogenic microorganisms, including the *Bacteroides* group, anaerobic cocci, the pyogenic cocci (including enterococci), and facultative gram-negative rods—which can be part of the vaginal and cervical flora in asymptomatic women[9-11]—complicates the interpretation of culture results from patients who develop fever after receiving antibiotic prophylaxis (Table I). The difficulties involved in obtaining meaningful uterine culture material from patients with the clinical diagnosis of endomyometritis have been discussed by several investigators.[12-15]

We previously reported the use of "double-lumen tubes" to decrease contamination from the cervix and vagina.[13] However, because these devices are more complex than regular swabs, inconsistent use during nocturnal fever evaluations has limited their value on our

Table I. Potentially pathogenic vaginal and cervical bacteria

Peptococcus sp.
Peptostreptococcus sp.
Bacteroides sp.
Escherichia coli
Proteus sp.
Klebsiella sp.
Enterobacter sp.
Streptococcus faecalis (Enterococcus)
β-Hemolytic group B *Streptococcus*
Clostridium sp.
Fusobacterium sp.
Staphylococcus aureus
Neisseria gonorrhoeae
Streptococcus pyogenes
Listeria monocytogenes
Gardnerella vaginalis

service. Therefore we have not been able to collect contamination-free culture data on all of our patients with a diagnosis of endomyometritis after cesarean section. Nevertheless, use of the double-lumen tubes has shown that our data are in excellent agreement with those reported by other investigators.[12-17] The most common bacterial species isolated from the infected uterus after cesarean section in our patients as in others are *Peptococcus, Bacteroides, Peptostreptococcus, Escherichia coli,* and enterococci, followed by other species of pyogenic cocci and facultative gram-negative bacilli (Table I). Such information regarding antibiotic failures should be useful in determining what an effective prophylactic antibiotic spectrum should include or protect against.

Status of host defenses

The physician must also consider the status of the patient's host defense mechanisms in choosing an antibiotic for prophylaxis. For example, the antimicrobial activity of amniotic fluid in our obstetric patients, who are primarily indigent, is generally poor and thus probably contributes to their relatively high rates of postpartum infections[18, 19] (Table II).

In contrast, most "private patients" at the same institution have excellent antimicrobial activity in the amniotic fluid and very low postpartum infection rates,[18, 19] which illustrates the importance of individualizing antibiotic use and selection (Table II).

The importance of interactions between antibiotics and host defenses has recently been recognized. For example, we and other investigators previously reported on the effects of antibiotics on many polymorphonuclear leukocyte functions, including chemiluminescence and chemotaxis, both of which are enhanced by piperacillin and clindamycin and depressed

Table II. Noncellular host defenses for amniotic fluid

	Clinic population	*Private population*
β-Lysin activity (units)	0-4	16-64
Zinc ion concentration (ppm)	15 ± 12	98 ± 15
Cationic proteins	0-+	+ + + +
Postpartum infection rate (%)	37-9	1

by some of the cephalosporins. Cefotaxime, for example, depresses these essential polymorphonuclear leukocyte functions[20-25] (Table III). Whether and how these in vitro data will correlate with significant clinical findings are still being studied. Nevertheless, the effect of an antimicrobial on polymorphonuclear leukocyte functions, particularly in indigent populations, may emerge as an important consideration in choosing a prophylactic antibiotic.

Discouraging bacterial resistance

The effects of antibiotic use on the emergence of resistant organisms should be thoroughly thought out. The use or misuse of antibiotics affects not only the individual patient but also both hospital and community environments.[26-31] Despite rigorous policies of infection control, house staff and nursing staff interact with and cross contaminate gynecologic oncology patients, benign disease gynecologic patients, and obstetric patients, thus enlarging the pool of potentially resistant microorganisms. Use of antibiotics that are strong inducers of β-lactamases should be carefully considered in view of their potential for increasing bacterial resistance to a variety of antimicrobials.[30] Adverse changes in microbial sensitivities and pathogenicities can occur as a result of prescribing practices. For example, the emergence of enterococci as postoperative pathogens certainly has been attributed to antibiotic use.[32, 33] More important, given the potential for cross contamination and the spread of resistant anaerobes (for example, *Bacteroides*) and gram-negative facultative bacilli (for example, *Pseudomonas, Proteus, Serratia*), physicians need to be increasingly aware and vigilant to help control these problems.

Clinical trials (University of California at Los Angeles experience)

Over the past several years, we prospectively evaluated the prophylactic efficacy of several β-lactam antibiotics including ampicillin, cefoxitin, cefazolin, ceftazidime, cefotaxime, and piperacillin in our indigent patients undergoing cesarean section under similar conditions.[5-7] All patients received 2 gm each of the

Table III. Effects of antibiotics on polymorphonuclear leukocyte functions: chemiluminescence and chemotaxis

	Chemotaxis (μm)*		*Chemiluminescence*	
	No antibiotic	*With antibiotic*	*No antibiotic*	*With antibiotic*
Piperacillin	79 ± 7	130 ± 10	1	1.9 ± 0.1
Clindamycin	79 ± 7	123 ± 8	1	1.8 ± 0.1
Cefoxitin	79 ± 7	75 ± 5	1	0.9 ± 0.1
Cefotaxime	79 ± 7	60 ± 8	1	0.7 ± 0.1
Tobramycin	79 ± 7	80 ± 6	1	1.1 ± 0.1
Gentamicin	79 ± 7	79 ± 9	1	1.0 ± 0.1

*Random migration was 37 ± 4 μm.

Table IV. Cost of failure of antibiotic prophylaxis in cesarean section patients

Maternal (isolation bed)	$447 × 5 days = $2235
Newborn observation bed	$614 × 5 days = $3070
Fever workup (mean)	$ 570
Clindamycin, gentamicin	$1251
Intravenous sets, pharmacy charges laboratory fees (creatinine, blood urea nitrogen, peak and trough)	$158 × 2 days = $ 316
Total	$7442

Table V. Cesarean section prophylactic study at the University of California at Los Angeles: Cost of antibiotic failure and subsequent therapy of infection per 100 patients

Piperacillin	$ 27,744
Cefoxitin	$ 81,078
Cefazolin	$139,056
Cefotaxime	$169,540
Ampicillin	$181,966

Table VI. Cost savings associated with piperacillin compared with other prophylactic antibiotic regimens in cesarean section

Piperacillin versus ampicillin	$154,222
Piperacillin versus cefotaxime	$141,796
Piperacillin versus cefazolin	$111,312
Piperacillin versus cefotaxime	$ 53,334

study drug when the umbilical cord was clamped and two additional doses of 2 gm each at 4-hour intervals.

Exclusion and efficacy criteria. Patients were excluded from the study if they had had any of the following: antimicrobial therapy within 7 days before entry into the study, a history of hypersensitivity to either penicillins or cephalosporins, renal and/or hepatic function tests substantially outside normal limits, or evidence of infection at the time of enrollment into the study.

Both direct and indirect criteria for the existence of postoperative morbidity were used to evaluate the efficacy of the antibiotic regimens: fever, duration of hospitalization, administration of systemic antibiotics after operation, and evaluation of wound healing and evidence of infection at the operative site. Febrile morbidity, as a manifestation of infection, was defined as an oral temperature ≥100.4° F on at least two occasions 4 to 6 hours apart, occurring more than 24 hours after operation. A satisfactory prophylactic response was defined as no bacterial or clinical infection at the operative site for 4 to 6 weeks after operation. An unsatisfactory prophylactic response was defined as the development of an infection at the operative site that required antibiotic administration during the initial 4- to 6-week postpartum period. Patient demographics and other variables such as the mean number of vaginal examinations, the mean time of ruptured membranes, the mean blood loss, the mean duration of operation, and the mean experience of the surgical team did not vary significantly among study groups.

Results

With the above criteria, the response to prophylaxis was considered effective in 98% of piperacillin-treated, 91% of cefoxitin-treated, 82% of ceftazidime-treated, 82% of cefazolin-treated, 80% of cefotaxime-treated, and 77% of ampicillin-treated patients. Statistically, there was a significant difference ($p = 0.035$) between the piperacillin and cefoxitin efficacy rates. Interestingly, however, there were no significant ($p < 0.05$) differences between the third-generation and first-generation cephalosporins.

To determine the extent to which the clinical success of prophylaxis could be translated into patient cost savings, the total mean costs of prophylactic failure in cesarean section were calculated. These patient costs were based on the minimum mean charges of treating in-

fections after cesarean section, including daily charges for hospital rooms (both maternal and normal nursery), laboratory tests and fees, and costs of drugs, pharmacy preparation, and intravenous equipment as of July 1, 1986 (Table IV). Significant but more difficult to determine than the above fees are the costs of potential medical-legal complications arising from patient anger and anguish associated with morbidity, as well as additional physician and nursing time to evaluate and treat the antibiotic failures. Given these data and our current costs, the estimates per 100 patients for failure of each drug are: ampicillin, $181,966; cefoxitin, $81,087; cefazolin, $139,056; cefotaxime, $169,540; and piperacillin, $27,744 (Table V).

Conversely, when the cost of 100 prophylactic failures for the most effective agent, piperacillin, was compared with the costs of ampicillin, cefotaxime, cephalothin, and cefoxitin, savings were $154,222 (piperacillin versus ampicillin), $141,796 (piperacillin versus cefotaxime), $111,312 (piperacillin versus cefazolin), and $54,334 (piperacillin versus cefoxitin), respectively (Table VI).

Pragmatically, the only correct prophylactic antibiotic regimen is whatever works best in the physician's own experience. However, if one's current prophylactic antibiotic regimen has a failure rate of 5% or higher, we recommend that consideration be given to an antibiotic with a broader spectrum of coverage, particularly of the enterococci and anaerobes.

Comment

In conclusion, the practicing obstetrician and gynecologist, before prescribing an antibiotic, must carefully consider, in addition to antimicrobial spectrum, the patient's allergies, the drug's potential toxicities, and the medical-legal implications of any prophylactic antibiotic failures. We must remember that drug costs include both acquisition costs and the costs for treating infections related to prophylactic failures. Diagnosis-related groups (DRGs), government regulations, and third-party payers have forced physicians to become more aware of financial considerations and to take a more active role in cost control. We are increasingly torn in several, often conflicting directions. On the one hand, we need to recognize the importance of and take part in containing and minimizing the costs of health care. On the other hand, we must never relinquish our responsibility to base medical decisions on the best interest of our patients. To ignore the cost containment issues involved in either the current DRGs situation or the malpractice crisis is to ignore reality. Nevertheless, physicians should not relinquish the control of therapy and the management of patients because of nonmedical, economic pressures.

Physicians should utilize antibiotic regimens that are both clinically effective and cost effective. Whenever possible, single-agent therapy rather than combination therapy should be used if clinical efficacy and safety can be demonstrated.

We wish to thank Dr. Elliot Landau of the Department of Biomathematics, University of California at Los Angeles, for his assistance in the statistical analysis of our data.

REFERENCES

1. Ledger WJ, Gee L, Lewis WP. Guidelines for antibiotic prophylaxis in gynecology. Am J Obstet Gynecol 1974; 121:1038.
2. Hilliard GD, Harris RE. Utilization of antibiotics for prevention of symptomatic post partum infections. Obstet Gynecol 1977;50:285.
3. Cunningham FG, Hauth JF, Strong JD, et al. Infectious morbidity following cesarean section: comparison of the treatment regimens. Obstet Gynecol 1978;52:656.
4. Hager WD, Williamson MM. Effects of antibiotics on women undergoing nonelective cesarean section in a community hospital. J Reprod Med 1983;28:687.
5. Ford LC, Hammill HA, Finegold SA, Lagasse LD, Lebherz TB. Prophylaxis of obstetrical and gynecological infections. In: Proceedings of the thirteenth international congress of chemotherapy, Vienna, Austria, August 28, 1983, vol 4:56.
6. Ford LC. A double-blind study of piperacillin versus cefoxitin. Infect Surg 1984;(suppl)27.
7. Ford LC. Indication for prophylaxis in cesarean section. Infect Surg 1984;30.
8. Ford LC, Hammill HA. Evaluation of piperacillin versus cefoxitin as a prophylactic agent in cesarean section. Chemotherapy 1985;4:746.
9. Galask RP, Larsen B, Ohm MJ. Vaginal flora and its role in disease entities. Clin Obstet Gynecol 1976;19:61.
10. Gorbach SH, Menda B, Thadepalli H, Keith C. Anaerobic microflora of the cervix in healthy women. Clin Obstet Gynecol 1973;117:1053.
11. Goplerud CP, Ohm MJ, Galask RP. Aerobic and anaerobic flora of the cervix during pregnancy and puerperium. Am J Obstet Gynecol 1976;126:858.
12. Ledger WJ, Gee CL, Pollin PA, Lewis WP, Sutter VL, Finegold SM. A new approach to patients with suspected anaerobic post partum pelvic infections: transabdominal uterine aspiration for culture and metronidazole for treatment. Am J Obstet Gynecol 1976;126:1.
13. Ford LC, DeJoseph J, Hammill HA, Lebherz TB. Evaluation of post partum culture techniques. J Obstet Gynecol 1983;4:97.
14. Stonick IJ, Stelluto M, Prystowsky H. Microbiology of the female genital tract. III. Comparative investigation of the cervical flora of parturients receiving either rectal or vaginal examinations. Am J Obstet Gynecol 1963;85:519.
15. Prevedourakis CN, Strigou-Charalabis B, Kaskarelis DB. Bacterial invasion of amniotic cavity during pregnancy and labor. Obstet Gynecol 1971;37:459.
16. Platt LD, Yonekura ML, Ledger WL. The role of anaerobic bacteria in postparum endomyometritis. Am J Obstet Gynecol 1979;135:814.
17. Gibbs RS, O'Dell TN, MacGregor RP, Schwarz RH, Morton H. Puerperal endometritis: a prospective microbiologic study. Am J Obstet Gynecol 1975;121:919.
18. Ford LC, Kasha W, Heins T, et al. Identification of beta-lysin as a zinc-dependent antibacterial protein in amniotic fluid. J Obstet Gynecol 1981;2:79.
19. Ford LC, Kasha W, Singer KL, et al. Antimicrobial activity of amniotic fluid beta-lysin and the influence of zinc ion. J Obstet Gynecol 1982;3:1.

20. Ford LC, Hammill HA. Effects of piperacillin, clindamycin, cefoxitin, cefoperazone, cefotaxime, tobramycin and gentamicin *in vitro* on polymorphonuclear leucocytes from leucopenic patients and from patients with severe pelvic inflammatory disease. Recent Adv Chemother 1985;3:2527.
21. Mandell LA. Effects of antimicrobial and antineoplastic drugs on the phagocytic and microbicidal function of the polymorphonuclear leukocyte. Rev Infect Dis 1982;4:683.
22. Spitzngel JK. Microbial interactions with neutrophils. Rev Infect Dis 1983;5:S806.
23. Fietta A, Bersani C, Grassi F, Mangiarotti P, Grassi GG. Effect of beta-lactam antibiotics on migration and bactericidal activity of human phagocytes. Antimicrob Agents Chemother 1983;23:930.
24. Oleske JM, de la Cruz A, Ahdieh H, et al. Effects of antibiotics on polymorphonuclear leukocyte chemiluminescence and chemotaxis. J Antimicrob Chemother 1983; 12(suppl):35.
25. Ueda Y, Saito A, Fukuda Y. Interaction of beta-lactam antibiotics and antineoplastic agents. Antimicrob Agents Chemother 1983;23:374.
26. Cuchural GJ, Tally FF, Storey JR, Malamy MH. Transfer of beta-lactamase-associated cefoxitin resistance in *Bacteroides fragilis*. Antimicrob Agents Chemother 1986;29:918.
27. Neu HC. Contribution of beta-lactamase to bacterial resistance and mechanisms to inhibit beta-lactamases. Am J Med 1985;79:2.
28. Phillips I. Beta-lactamase-producing penicillin-resistant gonococcus. Lancet 1976;2:656.
29. Eley A, Greenwood D. Beta-lactamases of type culture strains of the *Bacteroides fragilis* group and of strains that hydrolyse cefoxitin, latamoxef and imipenem. J Med Microbiol 1986;21:49.
30. Finland M. Emergence of antibiotic resistance in hospitals 1935-1975. Rev Infect Dis 1979;1:4.
31. Simpson IN, Herper PB, O'Callaghan CH. Principal beta-lactamases responsible for resistance to beta-lactam antibiotics in urinary tract infections. Antimicrob Agents Chemother 1980;17:929.
32. Barrall DT, Kenney PR, Slotman GJ, Burchard KW. Enterococcal bacteremia in surgical patients. Arch Surg 1985;120:57.
33. Leoung GS, Joseph P, Marzouk J, Mills J, Quintiliani R. Enterococcal superinfections. Infect Surg 1984;3(suppl): 644.

Questions from the audience

The following highlights are excerpted from two informative and spirited question-and-answer sessions, moderated by F. Gary Cunningham, M.D., Symposium Chairman.

Session 1

Q. Dr. Gall, could you reiterate the failure rates of the two prophylaxis regimens you used in your study—the triple-dose course of piperacillin versus the single dose?

Dr. Gall. The multi-dose regimen was successful in approximately 94% of patients compared with 87% for the single-dose group. Although these figures were not significantly different, I originally expected that they would be. That's the nice thing about a randomized, blinded, prospective trial: your own bias is irrelevant.

Moderator. Dr. Gall, what kind of infection rate could you have expected in your population if no perioperative prophylaxis had been administered?

Dr. Gall. On the basis of previous placebo studies, we might have expected a postoperative incidence of infection in the range of 45% to 50%.

Moderator. This is an important point because our background infection rate in high-risk patients at Parkland Hospital in Dallas is probably closer to 85% without perioperative prophylaxis. We should remember that most of these investigations are carried out in high-risk women from low socioeconomic groups in large urban hospitals. It is likely that single-dose prophylaxis would be much more effective for most of the private patients treated by the practitioners in our audience than for our patients in Chicago or Dallas.

Dr. Gall. In 1979, we published results of a study conducted at Duke University in which cefazolin was compared with placebo for patients undergoing cesarean section. Socioeconomic status, defined as public patient versus private patient, was one of the factors we looked at. We found a difference: the worst case scenario was placebo in a public patient, which resulted in a 51% infection rate. The same placebo in private patients resulted in a 40% incidence of infection. We don't really know whether these differences can be attributed to better protoplasm, or fewer bacteria, or other factors, but it may indeed be true that the shortest course of a prophylactic antimicrobial—the single dose—may be quite adequate for most private patients.

Q. Dr. Gall, could you comment on the significance, if any, of isolating mycoplasmas in so many of your patients who became infected?

Dr. Gall. Among infected patients, there was a much higher, albeit statistically nonsignificant, incidence of mycoplasmal colonization. Other investigators also have alluded to the possibility that the presence of mycoplasmas in the amniotic fluid might be a kind of marker for the development of clinical postoperative endometritis.

Moderator. Dr. Gall, did you isolate any enterococci in your postoperative cultures?

Dr. Gall. Yes, there were a few enterococci, but I only worry about them if they are isolated from the blood or from an immunocompromised patient.

Moderator. I agree with you, but I am worried about what happens with enterococci in 10 years. For instance, the "sin" of antimicrobial misuse is catching up with us in that we are now seeing methicillin-resistant *Staphylococcus aureus* catheter-associated infection in intensive care units. We are paying dearly for such past practices by treating patients with expensive intravenous vancomycin. It would behoove all of us to keep abreast of the literature and clinical evaluations of various drug regimens lest we risk using therapies that are becoming ineffective.

Session 2

Q. How do the third-generation cephalosporins penetrate into difficult-to-reach areas, or across biologic membranes, compared with penicillins?

Moderator. First of all, we've got to understand that a drug may be present in an abscess, for instance, without exerting any biologic activity. These things are actually becoming more difficult to study because more prophylaxis and more vigorous treatment with better drugs are making pelvic abscesses much less common than they used to be. Most of our data on abscess penetration therefore come from the rat model of Gorbach et al.

Q. I just find it surprising that a large cephalosporin-type molecule would be able to penetrate at all.

Dr. Hemsell. Penetration probably does not occur in undamaged meninges, for example. Inflamed meninges, on the other hand, apparently react differently.

Dr. Gall. The concentration of bacteria and the concentration of enzymes that break down inactive metabolites are several logs higher in an abscess. Even if an antibiotic gets in, it is in a much more hostile environment. The pH is lower and the polymorphonuclear leukocytes are probably not functioning well.

Moderator. In the final analysis, we have to look at whether these experimental results correspond in any way with clinical outcomes. Although we who are interested in research have to continue to get answers to questions about penetration, half-lives, serum levels, etc., the bottom line has to be, "does the drug work?" and "how long will it continue to be effective?"

Q. How should chorioamnionitis be managed?

Moderator. I think we can all agree that a woman with chorioamnionitis needs to be delivered. If she's not in labor already, labor should be induced. Moreover, we should treat these patients vigorously with some sort of intensive antimicrobial regimen during labor.

I am troubled, however, by those individuals who are recommending that delivery by the most expeditious route—cesarean section—be undertaken when chorioamnionitis is diagnosed. On the basis of my clinical experience, it seems that cesarean section in a laboring woman who has ruptured, leaking membranes, fever, and clinical chorioamnionitis can result in some pretty severe outcomes. The babies do not seem harmed by at least a trial of labor if appropriately monitored, and the mothers do better with vaginal deliveries.

Dr. Gall. At our institution, patients diagnosed as having chorioamnionitis and who have a temperature of ≥38° C are started on an antibiotic every 6 hours. We obtain a culture and induce labor or allow labor to proceed for 4 to 6 hours. If appropriate progress has not been made after a reasonable trial, the patient would probably be delivered of her infant by cesarean section. The antibiotic is continued for 72 hours post partum or for 24 hours after the patient becomes afebrile.

Moderator. I'd like to emphasize the fact that patients with chorioamnionitis who are delivered of their infants by cesarean section need intensive therapy with something. A few years ago, we used penicillin plus an aminoglycoside with poor results: a third of those women ultimately required hysterectomy within the first 10 days post partum for parimetrial phlegmons, broad ligament abscesses, and things of that nature. It's best to use the kind of "big gun" regimen—such as clindamycin and gentamicin, a cephalosporin with chloramphenicol, or piperacillin alone—that would be appropriate for the worst kind of pelvic sepsis. When a surgical delivery has been performed, chorioamnionitis presents a serious threat to maternal health.

In summary, we know that women with significant pelvic infections suffer some sort of breach of the epithelial defenses, either from surgical trauma or from infection with a primary invading organism (for example, *Neisseria gonorrhoeae, Chlamydia trachomatis*). We also know that these infections are polymicrobial.

The overuse of certain drugs, such as cephalosporins, has resulted in the emergence of bacterial strains that are resistant to some currently used regimens. Dr. Galask described a particularly interesting and important mechanism of gram-negative resistance—induction of β-lactamase production by exposure to certain β-lactam agents. It sounds theoretical, but we're already dealing with the clinical implications of this phenomenon in terms of increased resistance to cefoxitin.

In his discussion of therapy for serious female pelvic infections, Dr. Hemsell emphasized that the standard for any regimen—whether it is clindamycin plus gentamicin, or piperacillin—is a 90% to 95% success rate. I prefer to use single-agent therapy whenever efficacy is equivalent.

Enhancement or depression of polymorphonuclear leukocyte functions by antimicrobial agents, addressed by Dr. Ford, is a fascinating aspect of infection control. Moreover, he showed us how the higher overall costs of less effective prophylactic regimens underscore the importance and impact of using appropriate, effective surgical prophylaxis, particularly in high-risk patients.

While it still may be a theoretical problem, I am nevertheless disturbed by the finding that many women who receive perioperative prophylaxis have enterococci in the lower genital tract for 2 to 5 days after operation. It makes me think that piperacillin, which is quite effective against enterococci, will become more and more attractive as a prophylactic agent in the future.

In general, piperacillin is an ideal single agent for prevention and therapy of female pelvic infection. It has an excellent antibacterial spectrum, is clinically effective, and enjoys the safety of the penicillins. Its future value is not jeopardized by β-lactamase induction but rather is enhanced by its somewhat unique activity against enterococci. Finally, its cost is reasonable and competitive. I'd like to thank the faculty for their outstanding presentations and the audience for providing such stimulating discussion.

CORRESPONDENCE

Profound hypotension complicating magnesium therapy

To the Editors:

I agree with Bourgeois et al. (AM J OBSTET GYNECOL 1986;154:919-20) that significant hypotension with nontoxic magnesium levels is not a generally recognized side effect of magnesium sulfate therapy in preeclamptic patients. However, I disagree with the authors regarding their statement that there is no support for the possibility that severe hypotension can be caused by the concomitant administration of magnesium and promethazine.

Promethazine, a phenothiazine derivative, is a selective calmodulin inhibitor.[1] This pharmacologic action of the drug can promote vascular smooth muscle relaxation, vasodilatation, and hypotension by the following two mechanisms: (1) an indirect, β_2-sympathomimetic type of vasodilatation, caused by the calmodulin-mediated inhibition of phosphodiesterase,[1] and (2) a direct inhibition of vascular smooth muscle contractility by selective inhibition of calmodulin, the actin and myosin filament–coupling molecule.[2]

The concomitant administration of a calcium antagonist (magnesium sulfate) and a phosphodiesterase and calmodulin inhibitor substance (promethazine) can be responsible for the severe hypotension observed in the two patients described by the authors, despite their extensive clinical experience.

One should be aware of drug interactions, especially with magnesium sulfate in conjunction with β-sympathomimetic, vasodilator, calcium antagonist, and phenothiazine drugs.

At the Baragwanath Maternity Hospital, where 32,000 patients are delivered of infants annually and the incidence of hypertension in labor is 4.9%, we use hydroxyzine hydrochloride (Atarax), a nonphenothiazine antihistamine with meperidine, for all our patients on a regimen of magnesium sulfate therapy. We never recorded any significant episode of hypotension.

In conclusion, Bourgeois et al. alerted us to the dangerous interaction between magnesium and a phenothiazine derivative. In preeclamptic patients receiving magnesium sulfate therapy and parenteral analgesia, a non-phenothiazine antihistamine such as hydroxyzine hydrochloride should be used.

Emeric P. Frohlich, Dr.Med.
Department of Obstetrics and Gynaecology
University of the Witwatersrand
Johannesburg, South Africa

REFERENCES

1. Levin RM, Weiss B. Mechanism by which psychotropic drugs inhibit adenosine cyclic 3',5'monophosphate phosphodiesterase of brain. Mol Pharmacol 1976;12:581-9.
2. Adelstein RS. Calmodulin and the regulation of the actin-myosin interaction in smooth muscle and non-muscle cells. Cell 1982;30:349-50.

Fetal heart rate monitoring

To the Editors:

In their article last November (AM J OBSTET GYNECOL 1985;153:685-92), Fukushima et al. considered the limitations of autocorrelation in fetal heart rate monitoring. For antenatal fetal heart rate monitoring they recommended that the fetal electrocardiogram be recorded from the maternal abdomen. Their article ignored three important points.

First, at 27 to 33 weeks of gestation it is difficult, and often impossible, to detect the fetal electrocardiogram. This is the age at which critical decisions must be made whether to deliver prematurely by cesarean section those infants whose conditions are compromised as a result of preeclampsia or placental insufficiency.

Second, the new generation of ultrasound detectors, with autocorrelation and improved transducer design has made it possible to obtain reliable fetal heart rate traces over these critical weeks. The older peak detectors had an unacceptably high signal loss, now reduced to 4% at 28 weeks.[1] Signal loss can occur as a result of maladjustment or through fetal movement, hiccups, or arrhythmia, but it can be detected at once with a microprocessor on line to give a visible or audible signal. We have used the newer ultrasound fetal heart rate monitors for the past 4 years (about 5000 records a year) and have not failed to obtain a satisfactory antenatal record.

Third, the suggestion that each pulse interval must be measured for clinical purposes is incorrect. Fetal heart rate variation can be measured from the pulse intervals averaged over 3.75 seconds as the mean minute range (in milliseconds).[1] This provides a wide discrimination between normal mean values at 32 weeks (44 msec), the lower limit of normality (less than a third percentile, 31 msec), and the reduced variation symptomatic of hypoxemia (21 msec) before development of acidemia.

G. S. Dawes
C. W. G. Redman
Nuffield Department of Obstetrics and Gynaecology
John Radcliffe Hospital
Maternity Department
Headington, Oxford, England, OX3 9DU

REFERENCE

1. Dawes GS, Redman CWG, Smith JH. Improvements in the registration and analysis of fetal heart rate records at the bedside. Br J Obstet Gynaecol 1985;92:317-25.

Reply

To the Editors:

We thank Drs. Dawes and Redman for their comments on our article. The major cause of our differences seems to arise from a misunderstanding of the terms fetal electrocardiography and fetal heart rate variability. The ensuing discussion will clarify this situation.

A discussion of fetal electrocardiography is not meaningful unless the data acquisition system is defined. From the letter by Dawes and Redman we gather that they are using a single bipolar electrode system for external fetal electrocardiography (HP8040A). If so, we agree that the fetal electrocardiogram may not be recoverable during certain periods of gestation. The data acquisition system mentioned in our report is not a single bipolar electrode system, but as stated it is a multiple amplifier parallel group averaging system—in this case a 20-bipolar electrode system.[1] Because the study of fetal electrocardiogram with the use of this technique is in its early stages, we are not advocating its use but rather suggesting that this approach seems to be more reasonable than the mathematical and statistical manipulation of an imprecise biomechanical signal. The capabilities of this system are illustrated by Figs. 7 and 8 in the article, which were recorded during late labor.

Dawes and Redman view the new generation of ultrasound detectors that use autocorrelation as a definite improvement over the older, Doppler ultrasound–based systems and cite their experience in over 20,000 cases in which they were able to infallibly obtain satisfactory tracings. We do agree that the autocorrelated ultrasound signal produces fetal monitoring records of more pleasing appearance more often than the uncorrelated ultrasonic signal. However, the use of autocorrelation in fetal monitoring is a double-edged sword. If on the one hand we have a higher signal recovery rate and an improved tracing, we are never completely sure as to the origin of the signal unless the fetal electrocardiogram is recorded simultaneously or the fetal monitor is continuously monitored by trained personnel. Because the autocorrelation circuitry will reinforce any periodic noise originating from the fetus (e.g., fetal motion, hiccups), the mother (e.g., vessels, viscera), or the transducer-skin interface, a dangerous situation may result that may not be obvious to the clinician because of the cosmetically improved appearance of the fetal heart rate.[2,3]

It is clear from the letter by Dawes and Redman that the variability they are discussing is not the same as that based on the fetal electrocardiogram R-R intervals. Because we have no experience with extensive processing of relatively imprecise ultrasonic signals of this nature, we are in no position to discuss their findings. However, if the R-R intervals of high quality fetal electrocardiogram signals are adequately processed, there is little doubt that individual intervals are clinically important. If they are averaged for even a few seconds, it would not be possible to identify severe fetal heart arrhythmias such as premature ventricular contractions, sinus pause, or evanescent cardiac arrest. Additionally, because the degree of variability obtained with Doppler ultrasound differs with the degree of signal processing, the resultant electronic fetal monitor record reflects both physiologic data and instrumental manipulation. Unfortunately, at any given moment it is not possible to determine which component is the dominant factor. Hence, a clear distinction has to be made between fetal heart rate variability and electronic fetal monitor variability. The electronic fetal monitor is but a clinical tool; to fully use its capabilities, one must also be aware of its limitations.

Teiichiro Fukushima, M.D.
Ezra C. Davidson, Jr., M.D.
Edward H. Hon, M.D.
Department of Obstetrics and Gynecology
King-Drew Medical Center and
Charles R. Drew Postgraduate Medical School
Los Angeles, California 90059

REFERENCES

1. Hon EH. Noise reduction in fetal electrocardiography. Med Arts Sciences 1961;15:120; Obstet Gynecol 1983; 147:721.
2. Amato JC. Fetal heart rate monitoring. AM J OBSTET GYNECOL 1983;147:967.
3. Perry CP. Problem with fetal heart rate monitor. AM J OBSTET GYNECOL 1984;150:111.

Recurrent spontaneous abortion and human leukocyte antigen DRw8

To the Editors:

Through the years many authors have reported that human leukocyte antigens play an important role in the immunologic interaction between mother and fetus. We have studied human leukocyte antigen frequencies and sharing of human leukocyte antigens in 30 Japanese couples with early recurrent spontaneous abortion of unknown cause and 72 Japanese healthy couples with more than three children.

Table I shows the most interesting results of immunogenetic studies. Apparently, the female subject in patient groups has a significantly lower frequency of DRw8 antigen (0%), as compared with the controls (30.6%) ($x = -11.6$, $p < 0.0001$, corrected to $p < 0.003$). Furthermore, other previously published literature[1,2] showed few numbers of DRw8-positive women with recurrent spontaneous abortion.

Caucasian populations have a low gene frequency (2% to 3%) of DRw8 antigen as compared with the Japanese (15%). Thus it was unclear that with recurrent spontaneous abortion the number of DRw8-positive women was statistically low as compared with normal couples. The fact that there are few DRw8-positive women with recurrent spontaneous abortion seems to be meaningful.

Table I. Frequencies of DR human leukocyte antigens in Japanese control groups and patients who experienced recurrent spontaneous abortion

DR antigen	*Patient couples (n = 30) (%)*		*Control couples (n = 72) (%)*		*Random control (n = 425) (%)*
	Woman	*Man*	*Woman*	*Man*	
1	23.3	16.7	13.9	13.9	15
2	36.7	23.3	38.9	30.6	36
3	0	0	0	0	0
4	46.7	33.3	31.9	44.4	44
5	26.7	26.7	23.6	20.8	20
w6	16.7	23.3	22.2	13.9	19
7	0	0	0	0	0
w8	0*	26.7	30.6	29.2	29
w9	26.7	23.3	30.6	34.7	30
w10	0	0	0	0	0
w11(5)	6.7	13.3	8.3	5.6	7
w12(5)	20.0	13.3	15.3	15.3	13
w13(w6)	10.0	20.0	13.9	12.5	16
w14(w6)	10.0	3.3	8.3	1.4	3

*Female patient versus control female $p < 0.00015$, corrected to $p < 0.003$.

Conversely, in renal transplantation, Hendricks et al.[3] reported that the DRw6-positive recipients experienced rejection of their grafts much more rapidly than the DRw6-negative ones. Kashiwagi[4] reported that only DRw8-positive recipients had nearly 100% of 3-year survival despite human leukocyte antigen compatibilities.

In pregnancy and in renal transplantation, there may exist a low or high responder to isoantigens. The possibility exists that the DRw8-positive woman is more likely to induce maternal-fetal tolerance than the others. This new finding needs to be confirmed in future studies.

Sharing of human leukocyte antigens (A, B, C, DR, and DQ) between the couples is more common among patient groups than the normal ones. This result supports the findings of other authors.[5] Although this meaning remains obscure, it may be related to the production of blocking antibody.

Masaru Takamizawa, M.D.
Takeo Juji, M.D.
Hiroko Tsuneyoshi, Ph.D.
Mie Nieda, Ph.D.

Department of Blood Transfusion
Faculty of Medicine
University of Tokyo
Hongo 7-3-1
Bunkyo-ku
Tokyo 113, Japan

Tomoyuki Fujii, M.D.
Takashi Kawana, M.D.
Masahiko Mizuno, M.D.

Department of Obstetrics and Gynecology
Faculty of Medicine
University of Tokyo
Hongo 7-3-1
Bunkyo-ku
Tokyo 113, Japan

Reprint requests: Masaru Takamizawa, M.D., Department of Pediatrics, Tokyo University Branch Hospital, Mejirodai 3-28-6, Bunkyo-ku, Tokyo 113, Japan.

REFERENCES

1. Oksenberg JR, Persitz E, Amar A, Brautbar C. Maternal-paternal histocompatiblity: lack of association with habitual abortions. Fertil Steril 1984;42:389-95.
2. McIntyre JA, McConnachie PR, Taylor CG, Faulk WP. Clinical, immunologic, and genetic definitions of primary and secondary recurrent spontaneous abortions. Fertil Steril 1984;42:849-55.
3. Hendriks GFJ, Schreuder GM, D'Amaro J, van Rood JJ. The regulatory role of HLA-DRw6 in renal transplantation. Tissue Antigens 1986;27:121-30.
4. Kashiwagi N. The role of HLA antigens in transplantation. Nippon Rinsho 1984;42:786-802.
5. McIntyre JA, Faulk WP. Recurrent spontaneous abortion in human pregnancy: results of immunogenetical, cellular, and humoral studies. Am J Reprod Immunol Microbiol 1983;4:165-70.

Myocardial infarction in the puerperium

To the Editors:

In the article entitled "Acute myocardial infarction in the puerperium in patients receiving bromocriptine" (Am J Obstet Gynecol 1986;155:371-2) Iffy et al. reported on two cases of acute myocardial infarction in the puerperium and implicated bromocriptine as the causative agent. In my opinion the authors have not presented sufficient evidence to justify this conclusion.

Myocardial infarction is a rare but previously reported complication of the puerperium in patients who have not received bromocriptine.[1] Because coronary vasospasm and myocardial infarction are well established side effects of treatment with ergonovine,[2] the administration of this medication to one of the reported patients seems sufficient cause to explain the event. The authors theorize that bromocriptine may have been

contributory via an "inborn error of metabolism," which may have resulted in an inability of the patient to distinguish between hydrogenated and nonhydrogenated ergot alkaloids or an interaction between ergonovine and bromocriptine at the P_{450} microsomal enzyme system that may have led to potentiation of the action of ergonovine. Because the established pharmacologic action of bromocriptine is to cause vasodilatation and hypotension,[3] I find the authors' alternative explanation of its pharmacologic action interesting but totally speculative and without scientific support.

The second patient reported by Iffy et al. had an autopsy that revealed an occlusion of the anterior descending branch of the left coronary artery by thrombotic emboli, which resulted in the subsequent infarction. Because bromocriptine has no known effect on coagulation and thrombus formation, it is highly unlikely that the medication contributed to the clot formation in this patient. Other thrombotic complications of pregnancy are also seen and are thought to be caused by known hypercoagulable changes in the clotting mechanism that occur around the time of delivery.[4] This hypercoagulable condition may have contributed to the coronary artery thrombus formation in the aforementioned patient and in other cases of postpartum myocardial infarction previously reported.

Thank you for your consideration of these comments.

Harold N. Feldman, M.D.

Medical Services Department
Sandoz Pharmaceuticals
Dorsey Pharmaceuticals
Route 10
East Hanover, New Jersey 07936

REFERENCES

1. Bornstein A, Dalal P, Tischler J, et al. Acute myocardial infarction in a thirty-six-year-old postpartum female. Angiology 1984;35:591-4.
2. Taylor GJ, Cohen B. Ergonovine-induced coronary artery spasm and myocardial infarction after normal delivery. Obstet Gynecol 1985;66:821-2.
3. Kolloch R, Kobayashi K, De Quattro V, et al. Dopaminergic control of sympathetic tone and blood pressure: evidence in primary hypertension. Hypertension 1980;2:390-4.
4. Ciraulo DA, Markovitz A. Myocardial infarction in pregnancy associated with a coronary artery thrombus. Arch Intern Med 1979;139:1046-7.

Reply

To the Editors:

While focusing on the "proven pharmacologic action" of bromocriptine, Dr. Harold Feldman seems to overlook the fact that our article referred to the drug's untoward effects and not the predictable ones. Of the former, the product information by Sandoz Pharmaceuticals Corp.[1] quotes several dozens, including "signs and symptoms of ergotism." Because bromocriptine is an ergot derivative, this side effect should not be surprising. Accordingly, we do not think that our proposed interpretation of the pathologic mechanism of this phenomenon is "without scientific support."

We have searched the literature in vain for investigations concerning the combined effects of ergonovine and bromocriptine in the puerperium. If, as we suspect, the writer shares our ignorance in this regard, he cannot be satisfied more than we are about the absence of a potential relationship, whether simply additive or synergistic, since these two drugs are often used together post partum. It appears that we are not the only ones who are not reassured that the existence of a connection between the actions of Ergotrate and bromocriptine is "totally speculative." Since 1985, the manufacturers have found it fit to warn physicians that Parlodel rarely "may cause hypertension" and that "particular attention should be paid to patients . . . who have received within the preceding twenty-four hours other ergot alkaloids . . .."

With regard to the causation of coronary thrombosis, Dr. Feldman elects to emphasize hypercoagulability and disregards vasospasm. Our article made it clear that it was the latter effect that we considered potentially attributable to bromocriptine and not the former one. In support of this interpretation, our article referred to reported observations pertaining to the occurrence of episodes of vasospasm among patients taking bromocriptine.

We concur with Dr. Feldman that our article offered no sufficient evidence to prove a cause-effect relationship between the postpartum use of bromocriptine and the occurrence of myocardial infarction or other accidents deriving from vasospasm.[1] However, it appears to us that with regard to the routine prescription of Parlodel to healthy mothers for the purpose of ablactation, it is the safety of the drug that needs to be established beyond reasonable doubt and not its dangers.

Leslie Iffy, M.D.
Willem ten Hove, M.D.
Garry Frisoli, M.D.

University of Medicine and Dentistry of New Jersey
University Hospital
100 Bergen Street
Newark, New Jersey 07103

REFERENCE

1. Physicians' Desk Reference. 41st ed. Oradell, NJ: Medical Economics 1987:1783-4.

Venous thrombosis in patients with cardiac pacemakers and oral contraceptive use

To the Editors:

I have been interested in thrombosis and pulmonary embolism for the last 20 years. Also I have implanted about 2000 cases of permanent cardiac pacemakers, in both young and old, for the last two decades. I feel the conclusion drawn by Halub et al. (Thrombosis due to

permanent pacemaker and oral contraceptives. Halub MF, Robbie G, Deere LF. AM J OBSTET GYNECOL 1985;153:571-2) is not supported by the data. I have seen vascular thrombosis of the upper torso in patients who had pacemakers implanted and who were not using oral contraceptives. I have also seen the same problems in men and women without pacemakers. The overall incidence of venous thrombosis in cardiac pacemaker patients is less than 2%.[1] In fact, I have seen this phenomenon in only two patients in the last 500 implants; neither patient was using oral contraceptives.

I do agree with the authors' discussion and management of the case. It may be that the oral contraceptives aggravated the thrombosis and the authors preferred to take an alternate approach to contraception. However, there are not enough numbers to support the authors' conclusion that "oral contraceptive therapy is contraindicated in women with permanent venous pacemakers."

Gopal Rao, M.D.

Shadyside Hospital
5230 Centre Avenue
Pittsburgh, Pennsylvania 15232

REFERENCE

1. Chamorro H, Rao G, Wholey MH. Superior vena cava syndrome: a complication of transvenous pacemaker implantation. Radiology 1978;126:377-8.

Femininity—a problem(?)

To the Editors:

As a qualified gynecologist, I was asked to review the records of a local abortion center, to confirm that all local rules and regulations in Cleveland's abortion laws were not abridged and were properly observed. These were never broken, but I was deeply disturbed by the frequency of repeat abortions in some patients. In this group, repeat procedures were often done at 3- to 4-month intervals. Review of the counseling techniques and procedures showed they were proper but of no help in this group. The repeat pregnancies were not accidental.

While attempting to investigate the causes of the early repeat pregnancies, I was given a book stating that pregnancy was a major method that confirmed to the patient that she possessed full femininity. However, because of varying social or economic reasons, the pregnancy was not carried to term. I tried to get additional data from the author but failed to get any response. Shortly thereafter, I restudied a scientific exhibit on premenstrual tension that I had presented to The American College of Obstetricians and Gynecologists in 1956. At that time, I reported that three of four patients had symptoms of the disorder but only one in 10 complained. In an attempt to answer this problem, I called a meeting of the entire group of patients. The most frequent answer was, "Doctor, I am a woman and this is the price we all pay for being women!" Therefore we have two aspects of femininity. One accepts the symptoms of the premenstrual syndrome but continues to work. The other gets pregnant and then aborts. If the patient can accept either phase, this condition is acceptable, but if she does not, this then becomes a real problem.

Thus we have two groups of women, almost two generations apart, in degree, accepting the same concern. Recently, I discussed this problem briefly with Dr. Luella Klein, the recent Past President of The American College of Obstetricians and Gynecologists who is actively fighting the occurrence of unwanted pregnancies. She told me that this is one of the greatest problems encountered and that many groups are working very hard in attempts to take these and similar thoughts away from women. Should she and her associates be successful, we may have the answer on how to more fully prevent the truly unwanted pregnancy.

I pray and hope that all physicians will understand the problem I have presented and will do their very best to overcome it, assuring women that unwanted pregnancies are not a desirable answer to femininity.

Eduard Eichner, M.D.

3333 Daleford Road
Shaker Heights, Ohio 44120

Definitions of sensitivity, specificity, false negative results, and false positive results

To the Editors:

The article by Kogon et al. (Kogon DP, Oulton M, Gray JH, et al. Amniotic fluid phosphatidylglycerol and phosphatidylcholine phosphorus as predictors of fetal lung maturity. AM J OBSTET GYNECOL 1986;154: 226-30) suggests that phosphatidylglycerol and phosphatidylcholine phosphorus are reliable indicators of fetal lung maturity. However, they have incorrectly defined sensitivity, specificity, false positive rate, and false negative rate. Sensitivity is defined as the percent of individuals with a disorder in which a test predicts for this disorder. In this case, the disorder is fetal lung immaturity and the test is the determination of phosphatidylglycerol. The absence of phosphatidylglycerol is the predictor of disease. Therefore the sensitivity should be 78.3%. The improper definition of sensitivity led the authors to incorrectly state their specificity as 78.3%. Specificity is defined as the percent of individuals free of disease with a negative test (that is, the presence of phosphatidylglycerol). Therefore 592 out of 620 patients had a negative test, for a specificity of 95.5%.

In addition, the authors have confused the false positive rate with the probability of a false positive result. The false positive rate is the percent of false positives out of the *total* number of *positive* results. In this study, there were 28 false positive results out of 64 positive results or 43.7%. The false negative rate is defined as the percent of false negative results out of the total number of negative results; 1.7% in this study.

Proper use of the definitions of sensitivity and specificity will also lead to a reversal in the positive predictive value and the negative predictive value; the positive predicted value should be 56.3% and the negative predicted value should be 98.3%.

Although the conclusions, with the exception of the false negative rate and the false positive rate, remain basically intact, strict adherence to the definitions of sensitivity and specificity will eliminate errors in the construction of a binomial table and facilitate comparison among investigators.

Mark D. Kolins, M.D.

Department of Pathology
Detroit Riverview Hospital
7733 E. Jefferson
Detroit, Michigan 48214

Haemophilus influenzae infection and midtrimester abortion

To the Editors:

It is always dangerous to state that "this is the first case" or "this is the only case" when reporting an unusual event. Certainly we have all missed one or even a series of examples of the same phenomenon. An example of such a situation pertains to the report by Winn and Egley concerning *Haemophilus influenzae* chorioamnionitis (Winn HN, Egley CC. Acute *Haemophilus influenzae* chorioamnionitis associated with intact amniotic membranes. AM J OBSTET GYNECOL 1987;156:458). We reported a nearly identical case in 1979,[1] which demonstrated a systemic infection followed by intrauterine infection and delivery in the second trimester.

Marvin S. Amstey, M.D.
Edward Ogden, M.D.

Highland Hospital
South Avenue at Bellevue Drive
Rochester, New York 14620

REFERENCE

1. Ogden E, Amstey M. *Hemophilus influenza* septicemia and midtrimester abortion. J Reprod Med 1979;22:106-8.

Books received

Alcohol and Brain Development. Edited by James R. West, Ph.D. 441 pages, illustrated. New York, 1986, Oxford University Press. $55.00.

Biostatistics in Clinical Medicine. Second Edition. Joseph A. Ingelfinger, Frederick Mosteller, Lawrence A. Thibodeau, and James H. Ware. 339 pages, illustrated. New York, 1987, Macmillan Publishing Co., Inc.

Breasts, Bottles and Babies. Valerie Fildes. 462 pages, illustrated. Edinburgh, Scotland, 1986, Edinburgh University Press. No price listed.

Childbearing: A Book of Choices. Ruth Watson Lubic. 326 pages. New York, 1987, McGraw-Hill Book Company. $18.95.

Clinical Genetics Handbook. National Genetics Foundation, Inc. Ruth Y. Berini and Eva Kahn. 386 pages. Oradell, New Jersey, 1987, Medical Economics Books. No price listed.

Current Concepts in Gynecologic Surgery. Edited by James L. Breen and Howard J. Osofsky. Volume 3. 380 pages, illustrated. Baltimore, 1987, William & Wilkins. $88.95.

Detection, Prevention and Management of Urinary Tract Infections. Calvin M. Kunin. Fourth Edition. 448 pages, illustrated. Philadelphia, 1987, Lea & Febiger. No price listed.

Diagnosis and Management of the Fetus and Neonate at Risk: A Guide for Team Care. Fifth Edition. Martin L. Pernoll, Gerda I. Benda, and S. Gorham Babson. 365 pages, illustrated. St. Louis, 1986, The C. V. Mosby Company. No price listed.

Diagnostic Imaging of Infertility. Alan C. Winfield and Anne Colston Wentz. 237 pages, illustrated. Baltimore, 1987, Williams & Wilkins. $57.95.

Human Milk in Infant Nutrition and Health. Edited by R. Rodney Howell, Frank H. Morriss, Jr., and Larry K. Pickering. 334 pages, illustrated. Springfield, Illinois, 1986, Charles C Thomas, Publisher. $49.00.

In Vitro Fertilisation—Past, Present, Future. Edited by S. Fishel and E. M. Symonds. 275 pages, illustrated. Oxford, 1986, IRL Press Limited. No price listed.

Internal Medicine. Jay H. Stein, Editor in Chief. Second Edition. 2416 pages, illustrated. Boston, 1987, Little Brown & Company.

Menopause: Physiology and Pharmacology. Daniel R. Mishell, Jr. 368 pages, illustrated. Chicago, 1987, Year Book Medical Publishers, Inc. No price listed.

Neurological Assessment During the First Year of Life. Claudine Amiel-Tison and Albert Grenier. 198 pages, illustrated. New York, 1986, Oxford University Press.

On Moral Medicine: Theological Perspectives in Medical Ethics. Edited by Stephen E. Lammers and Allen Verhey. 658 pages. Grand Rapids, Michigan, 1987, William B. Eerdmans Publishing Company.

Pain Management: Assessment and Treatment of Chronic and Acute Syndromes. Edited by Wen-hsien Wu. Leon G. Smith, Consulting Editor. 397 pages, illustrated. New York, 1987, Human Sciences Press, Inc. No price listed.

Practical Aspects of gynaecourology. Proceedings of the 9th Annual Meeting of IUGA. A. Tanko, I. Berbik, and E. Petri. 470 pages. Budapest, Hungary, 1986, Publishing House of the Hungarian Academy of Sciences. $49.00.

A Practical Guide to Ultrasound in Obstetrics and Gynecology. Eric E. Sauerbrei, Khanh T. Nguyen, and Robert L. Nolan. 195 pages, illustrated. New York, 1986, Raven Press. $24.50.

Risking the Future: Adolescent Sexuality, Pregnancy, and Childbearing. Volume 1. Cheryl D. Hayes, Editor. 337 pages. Washington, D. C., 1987, National Academy Press. $21.95.

Teenage Pregnancy in Industrialized Countries. A Study Sponsored by the Alan Guttmacher Institute. Elise F. Jones, et al. 310 pages, illustrated. New Haven, Connecticut, 1986, Yale University Press.

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

Nineteenth Memphis Conference on the Mother, Fetus, and Newborn, September 17-18, 1987, The Peabody Hotel, Memphis, Tennessee. Sponsored by The University of Tennessee, Memphis, College of Medicine and Regional Medical Center at Memphis. For further information contact: Office of Continuing Medical Education, 800 Madison Ave., Memphis, TN 38163. Tel.: (901) 528-5547.

Pediatric and Adolescent Gynecology Postgraduate Course, October 15-16, 1987, The Inn at Children's, Boston, Massachusetts. Co-sponsored by The Children's Hospital of Boston and Harvard Medical School, Department of Continuing Education. Approximately 14 hours credit, Category I. For information contact: Dr. S. Jean Emans, The Children's Hospital, 300 Longwood Ave., Boston, MA 02115.

International Symposium on First Trimester Genetic Diagnosis and Therapy: Current Status and Future Prospects, September 17-19, 1987, Hilton Hotel and Towers, Chicago, Illinois. Sponsored by the Medical and Reproductive Genetics Section, Eugene Pergament, Director, Department of Obstetrics and Gynecology, Illinois Masonic Department of Obstetrics and Gynecology. For information contact: Janet Dalzell, Illinois Masonic Medical Center, 836 West Wellington St., Chicago, IL 60657. Tel.: (312) 883-7045.

Specialty Review in Obstetrics and Gynecology: Practical Aspects, October 4-10, 1987, Chicago, Illinois. Sponsored by The Cook County Graduate School of Medicine. For additional information contact the Registrar's Office. Toll-free number: Illinois 1(800) 621-4649; outside Illinois 1(800) 621-4651.

The American Board of Laser Surgery announces its 2nd Certifying Examination on Saturday, October 10, 1987, at the University of California Medical Center, San Francisco, California. Candidates may obtain information and applications from the President of the Board, Stanley Stellar, M.D., St. Barnabas Medical Center, Livingston, NJ 07039, or the Assistant to the President, Mary Cayton, R.N., 3201 So. 16th St., Suite 2006, Milwaukee, WI 53215.

Premenstrual States: The Psychoanalytic/Psychobiologic Interface, October 17, 1987, Society for Ethical Culture Meeting House at Central Park West and 64th St., New York, New York, from 8:30 AM to 5:00 PM. Sponsored by the Columbia University Center for Psychoanalytic Training and Research in collaboration with the Association for Psychoanalytic Medicine. For further information contact: Columbia University Center for Psychoanalytic Training and Research, 722 West 168th St., New York, NY 10032. Tel.: (212) 927-5000.

National Abortion Federation wishes to announce its Fall Risk Management Seminar, "Beyond Sixteen Weeks: Practical Considerations for Quality Care," September 20-22, 1987, Four Seasons Olympic Hotel, Seattle, Washington. For more information, please contact NAF. Tel.: (202) 546-9060.

OBSTETRICIAN/ GYNECOLOGIST

An immediate opening exists for two fully qualified, or eligible, Obstetrician/Gynecologist in a fully accredited 220 bed acute care hospital. This regional hospital is located in northern Nova Scotia serving a referral base of 50,000. Area provides excellent schools, and year round recreational and sports facilities. In hospital office space available with full access to patient records.

Please submit application with three references to:

Administrator
Aberdeen Hospital
835 East River Road
NEW GLASGOW, Nova Scotia
B2H 3S6

OB/GYN

The perfect opportunity for a pair of obstetricians/Gynecologists to enjoy the benefits of joining a 165 physician multi-specialty group practice and of practicing in a small primary care group setting. Growing university town setting in Central Illinois offers pleasant mid-size community environment. Join this thriving 6 physician primary care branch and meet immediate needs. Liberal fringe benefits and salary lead to equal ownership in overall organization. Malpractice coverage provided through Clinic's self-insurance policy. Send CV and letter of interest to Head-Ob/Gyn Department; Carle Clinic, 602 West University Avenue; Urbana, IL 61801.

OB/GYN

Central Oklahoma Medical Group is one of Oklahoma City's largest mutli-specialty group practices with 40,000 prepaid members as well as traditional fee-for-service patients. We are currently seeking a BC/BE or completing residency in 1988 staff OB/GYN. Attractive salary and benefits package as well as opportunity to work with well trained partners in one of our modern facilities. Hospital is located across the street and is nationally recognized for it's progressiveness and quality of care. Contact:

Lois Goldstein
Central Oklahoma Medical Group
3330 N.W. 56th
Oklahoma City, OK 73112
405-945-1311

GYNECOLOGIC ONCOLOGIST

The Department of Obstetrics and Gynecology of the College of Physicians and Surgeons of Columbia University is accepting applications from Board Eligible or Board Certified gynecologic oncologists to join its Division of Gynecologic Oncology. We are interested in applicants with academic career interest. We offer an exciting environment for patient care, teaching and research.

Interested persons should direct inquiries and curriculum vitae to:

DANIEL H. SMITH, M.D.
COLUMBIA UNIVERSITY
COLLEGE OF PHYSICIANS AND SURGEONS
DEPARTMENT OF OBSTETRICS AND GYNECOLOGY
630 WEST 168TH STREET
NEW YORK, NEW YORK 10032
(212) 305-3410

Columbia University is an equal opportunity employer.

OBSTETRICIAN/ GYNECOLOGIST

Board eligible or certified general obstetrician/gynecologist to practice at university medical school affiliated hospital in nearby Hamilton, NY and engage in private practice. The community is an excellent environmental and intellectual setting and serves as the home for Colgate University. A medical college appointment at SUNY Health Sciences Center, Syracuse will be offered.

Salary guaranteed for the first year at $130,000 with other additional benefits.

Please submit curriculum vitae:

Michael S. Baggish, M.D.
Professor and Chairman
736 Irving Avenue
Suite 308, West Tower
Syracuse, New York 13210

PACIFIC NORTHWEST

Expanding Practice Seeks OB/Gyn

Ground floor opportunity with BC/BE women.

- Diverse urban clientele.
- Affiliation with major hospital.
- First year income guarantee.
- New family-oriented delivery facility.
- Malpractice, other benefits.
- Partnership potential.

For detailed fact sheet, contact:

THE FRIEDRICH GROUP
9284 Ferncliff, N.E.
Bainbridge Island, WA 98110
(206) 547-7850 or (206) 842-5248

OBSTETRICIAN-GYNECOLOGIST

Board Eligible/Board Certified OB-GYN to join four physician Department in 39 physician fee for service multispecialty group in a city of 70,000 located adjacent to the Rocky Mountains. Large drawing area, outstanding variety of year-round recreational lifestyle. Guaranteed minimum income, malpractice, educational benefits and early partnership. For further details, please forward C.V. to:

Gordon K. Phillips, M.D.
1400 29th St. So.
P. O. Box 5012
Great Falls, MT 59403

The Johns Hopkins Medical Institutions
Baltimore, Maryland

THE 29th ANNUAL
EMIL NOVAK
MEMORIAL COURSE:

Gynecology, Gynecologic Pathology, Endocrinology and High Risk Obstetrics
(including microscopic histopathology)

October 26 – 30, 1987

Microscopic Sessions Daily

For further information and registration contact:
Carlita M. Kearney
Program Coordinator
Office of Continuing Education
Room 19 Turner, 720 Rutland Avenue
Baltimore, Maryland 21205
(301) 955-3168

AMA Accredited

Maternal-Fetal Medicine

Board certified/eligible PERINATOLOGISTS required for large volume obstetric services (3,000-6,000 deliveries per year).

Opportunities exist both to join and create high risk obstetric services. The Southern California Permanente Medical Group has some openings for exclusive perinatal practice. However, not all areas have the volume of high risk patients to offer such exclusive practice. Perinatologists who wish to perform other aspects of obstetric and gynecologic practice may do so. University affiliations and clinical research is encouraged.

Excellent benefits are offered including liability coverage, medical and dental coverage, vacation and sick leave, continuing education and retirement program. Partnership consideration available after two years full time employment.

Please write, including your curriculum vitae and three professional references to:

KAISER PERMANENTE
SOUTHERN CALIFORNIA
PERMANENTE MEDICAL GROUP

William G. McCormick, M.D.
Physician Recruitment
Dept. 41L1
Walnut Center
Pasadena, CA 91188-8854
(714)829-5365
Equal Opportunity Employer M/F/H

OB/GYN

WANTED: Obstetrician/Gynecologist, Board Certified or eligible physician to join existing 3 member OB/GYN Department in 22 member multi-specialty group practice in the Lakes Region of central N.H. Position offers an outstanding professional, financial, and life-style opportunity. Respond with C.V. to Alan Awrich, M.D., Laconia Clinic, 724 Main Street, Laconia, NH 03246.

REPRODUCTIVE ENDOCRINOLOGY FELLOWSHIP
Louisiana State University School of Medicine in New Orleans

The Department of Obstetrics and Gynecology offers an accredited fellowship in Reproductive Endocrinology beginning July 1988. The fellowship is a two year clinical and research oriented program. Applicants must have completed an approved residency in Obstetrics and Gynecology by June 1988. The clinical services are located at the Charity Hospital and Southern Baptist Hospital in New Orleans. Research opportunities include ongoing programs in andrology, and fertility and sterility problems including an IVF/ET service as well as basic research involving the rodent, primate and pig models. Both clinical and laboratory research are encouraged and supported by local, regional, national and international agencies. Applications, including curriculum vitae, bibliography, and the names and addresses of at least three references should be sent before December 1987 to:

Krishna B. Singh, M.D.
Associate Professor and Chief
Section of Reproductive Endocrinology
OR
Charles A. White, M.D.
Professor and Head
Department of Obstetrics and Gynecology
Louisiana State University School of Medicine
1542 Tulane Avenue
New Orleans, LA 70112
An Equal Opportunity Employer

REPRODUCTIVE ENDOCRINOLOGIST

Marshfield Clinic, a 250 physician multispecialty private group practice in Central Wisconsin is seeking a Fellow trained BC/BE Reproductive Endocrinologist to join its 11 member Department of OB/GYN which includes a Perinatologist and Gynecologic Oncologist. The Clinic serves as a major regional referral center and provides a comprehensive spectrum of existing supporting specialties and services. An adjacent 524 bed acute care teaching hospital includes a Level III Perinatal Center and a 24 bed Neonatal Unit. Teaching and research opportunities also exist. Please send curriculum vitae and the names of several references to:

Mr. John P. Folz
Director-Patient Services
1000 North Oak Avenue
Marshfield, WI 54449
or call collect at
(715) 387-5181

Marshfield Clinic

PACIFIC NORTHWEST

Busy High Risk Practice

Join well-established, 25 person multi-specialty group in Tacoma.

Two blocks from Tertiary Center

Perform micro and laser surgery, laparoscopy, colposcopy

Initial income guarantee

Generous benefit package

Partnership potential

For detailed fact sheet, contact:

The Friedrich Group, Inc.
9284 Ferncliff Northeast
Bainbridge Island, WA 98110
(206) 547-7850 or (206) 842-5248

Faculty Position Available:

GYNECOLOGIC-ONCOLOGIST

The Department of Obstetrics and Gynecology at the Medical College of Ohio is accepting applications for a full-time position as oncologist to supervise a busy oncology service. Will be responsible for tumor program, including patient care, student and resident education. Attractive living area. Salary and academic rank commensurate with candidate's training and experience. Must be Board eligible/Board certified. Send inquiries and curricula vitae to:

John L. Duhring, M.D.
Chairman and Professor
Department of Obstetrics and Gynecology
Medical College of Ohio
C.S. 10008, Toledo, OH 43699

The Medical College of Ohio practices equal opportunity in education and employment.

Michael Reese Health Plan

A LARGE HOSPITAL-BASED HMO

has openings for the following full-time position:

Obstetrician Gynecologist

THE MICHAEL REESE HEALTH PLAN, a well-established and rapidly-expanding, federally-qualified HMO seeks Board Certified/Eligible Obstetrician/Gynecologists.

THIS POSITION entails both outpatient and hospital practice as well as medical students and house staff teaching.

REMUNERATION is high and competitive with academic and fee-for-service practice markets. Fringe benefits are generous.

INTERESTED APPLICANTS should send Curriculum Vitae in confidence to:
Dr. Stephen Contro, Medical Director, MICHAEL REESE HEALTH PLAN, 2545 Martin Luther King Drive, Chicago 60616

an equal opportunity employer m/f/h

OBSTETRICIAN/ GYNECOLOGIST

The Department of Obstetrics and Gynecology of Tufts University School of Medicine and St. Margaret's Hospital for Women is seeking Generalists to join an expanding, high volume practice. Candidates should have an active interest in clinical practice, and resident and medical school education. Several positions are available. Interested candidates should contact:

Stephen Curry, M.D.
Professor and Chairman of the
Department of Obstetrics and Gynecology
Tufts University School of Medicine
and the New England Medical Center
750 Washington St.
Boston, MA 02111
Telephone: 617-956-6063

OBSTETRICIAN/ GYNECOLOGIST

Marshfield Clinic, a multispecialty private group practice of 250 physicians, is seeking an additional Obstetrician/Gynecologist to join its expanding 11 member OB/GYN Department. Large regional referral center practice with high risk program. Adjacent 524 bed acute care hospital includes a Level III Perinatal Center and a 24 bed Neonatal Unit. Teaching and research opportunities also exist. Send curriculum vitae to:

Mr.John P. Folz
Direct-Patient Services
1000 North Oak Avenue
Marshfield, WI 54449
or call collect at
(715) 387-5181



MarshfieldClinic

NORTH SUBURB OF CHICAGO

Position available with 45-person multi-specialty group practice. OB/Gyne needed to share responsibility for busy practice with two OB/Gynes. Competitive salary and benefits. Contact: Jose Kogan, M.D., Medical Director, North Suburban Clinic, Ltd., 4801 Church Street, Skokie, IL 60077.



KAISER PERMANENTE

Good People. Good Medicine.

Maternal-Fetal Medicine

BC/BE Perinatologist wanted to join another subspecialist in a large multispecialty HMO located in the San Francisco-Oakland Bay Area.

Responsibilities include clinical service in two tertiary referral centers with 6,000 deliveries/year; consultations in high risk patients, genetic testing, ultrasound, resident teaching, and perinatal outreach programs. CVS experience desirable.

Each institution with full NICU capabilities with total of six neonatologists. Oakland facility with approved residency program (12 residents), oncologist, endocrine-infertility subspecialists, Level II ultrasound capabilities and antenatal testing unit.

Excellent benefits including competitive salary, malpractice insurance, medical and dental coverage, vacation and sick leave, pension and retirement programs. University affiliation and clinical research available.

Please write or call, including your curriculum vitae and three references:

Victor Chin, M.D., FACOG
Chief, Department of Obstetrics & Gynecology
The Permanente Medical Group, Inc.
280 West MacArthur Blvd.
Oakland, CA 94611
415 428-7340
or Lawrence Newman, M.D., FACOG
Perinatologist
415 428-7087

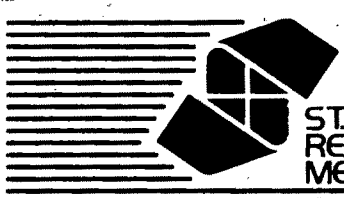
OBSTETRICIAN/ GYNECOLOGIST

The largest Medical Center is the State of Kansas offers an outstanding opportunity for physicians in a clean, family oriented community of 300,000 people.

Excellent salary and benefits. Guaranteed malpractice coverage and income. Consideration for other assistance negotiable.

Please contact:

Jim Hoffman, M.D.
Vice President, Medical Affairs
St. Francis Regional Medical Center
929 N. St. Francis
Wichita, KS 67214
(316) 268-8641



ST. FRANCIS REGIONAL MEDICAL CENTER
WICHITA, KANSAS

EQUAL OPPORTUNITY EMPLOYER

MOSBY Medical Journals

Medical specialty publications bringing important medical literature to over 205,000 worldwide subscribers throughout the year. MOSBY journals provide information progressive physicians need to maintain a continuing education program in their specialty areas. **SAMPLE COPIES of journals are available.**

* **THE JOURNAL *of* ALLERGY *and* CLINICAL IMMUNOLOGY**
Monthly. Institutions: $92.00; Individuals: $45.00; Students, residents: $32.00. Official publication of the American Academy of Allergy and Immunology.

CLINICAL NUTRITION
Bimonthly. Institutions: $39.00; Individuals: $39.00; Students, residents: $21.00.

* **CLINICAL PHARMACOLOGY *and* THERAPEUTICS**
Monthly. Institutions: $113.00; Individuals: $65.00; Students, residents: $45.00. Official publication of the American Society for Pharmacology and Experimental Therapeutics and the American Society of Clinical Pharmacology and Therapeutics.

* **JOURNAL *of* THE AMERICAN ACADEMY *of* DERMATOLOGY**
Monthly. Institutions: $104.00; Individuals: $57.00; Students, residents: $36.00. Official publication of the American Academy of Dermatology.

* **THE JOURNAL *of* HAND SURGERY (American Volume)**
Bimonthly. Institutions: $85.00; Individuals: $38.50; Students, residents: $25.00. Official publication of the American Society for Surgery of the Hand.

* **AMERICAN HEART JOURNAL**
Monthly. Institutions: $98.00; Individuals: $51.00; Students, residents: $33.00.

* **HEART & LUNG: THE JOURNAL *of* CRITICAL CARE**
Bimonthly. Institutions: $68.00; Individuals: $21.00; Students, residents: $16.00.

* **AMERICAN JOURNAL *of* INFECTION CONTROL**
Bimonthly. Institutions: $66.00; Individuals: $19.75; Students, residents: $15.00. Official publication of the Association for Practitioners in Infection Control.

* **THE JOURNAL *of* LABORATORY *and* CLINICAL MEDICINE**
Monthly. Institutions: $109.00; Individuals: $62.00; Students, residents: $35.00. Official publication of the Central Society for Clinical Research.

* **AMERICAN JOURNAL *of* OBSTETRICS *and* GYNECOLOGY**
Monthly. Institutions: $102.00; Individuals: $59.50; Students, residents: $40.00. Official publication of the American Board of Obstetrics and Gynecology, The American Gynecological and Obstetrical Society, plus 32 regional societies.

* **OTOLARYNGOLOGY—HEAD *and* NECK SURGERY**
Monthly. Institutions: $115.00; Individuals: $70.00; Students, residents (non-Academy members): $45.00; Residents (Academy members): $20.00. Official publication of the American Academy of Otolaryngology–Head and Neck Surgery, the American Academy of Otolaryngic Allergy, and the American Neurotology Society.

* **THE JOURNAL *of* PEDIATRICS**
Monthly. Institutions: $95.00; Individuals: $47.50; Students, residents: $30.00.

* **SURGERY**
Monthly. Institutions: $97.00; Individuals: $49.50; Students, residents: $32.00. Official publication of the Society of University Surgeons, Central Surgical Association, and the American Association of Endocrine Surgeons.

* **THE JOURNAL *of* THORACIC *and* CARDIOVASCULAR SURGERY**
Monthly. Institutions: $118.00; Individuals: $70.00; Students, residents: $45.00. Official publication of the American Association for Thoracic Surgery and the Western Thoracic Surgical Association.

* **JOURNAL *of* VASCULAR SURGERY**
Monthly. Institutions: $98.00; Individuals: $57.00; Students, residents: $35.00. Official publication of the Society for Vascular Surgery and the International Society for Cardiovascular Surgery (North American Chapter).

THE JOURNAL *of* HEART TRANSPLANTATION *new*
Bimonthly. Institutions: $60.00; Individuals: $40.00; Students, residents: $25.00. Official publication of the International Society for Heart Transplantation.

* **Included in Index Medicus** AJS-078

A Tradition of Publishing Excellence

Subscription rates quoted are for USA only. International rates (surface and airmail delivery) available upon request. All subscriptions are for one-year terms. Subscriptions may begin at any time.

THE C.V. MOSBY COMPANY
Journal Circulation Department
11830 Westline Industrial Drive
St. Louis, Missouri 63146

In the USA, call toll-free:
1-800-325-4177, ext. 351
In Missouri, call collect:
(314) 872-8370, ext. 351

INDEX TO ADVERTISERS

[redacted]ories
..... 26A,27A,28A

[redacted] Hospital
[redacted]portunity Available 33A

[redacted]rle Clinic
Opportunity Available 33A

[redacted]entral Oklahoma Medical Group
Opportunity Available 33A

Columbia University, College of Physicians and Surgeons
Opportunity Available 33A

FilmTec
Informed Consent Systems 19A

Friedrich Group, The
Opportunity Available 34A,36A

John Hopkins Medical Institutions
Course 34A

Kaiser Permanente
Opportunity Available 35A

Laconia Clinic
Opportunity Available 35A

Louisiana State University School of Medicine
Fellowship 35A

Marshfield Clinic
Opportunity Available 45A,37A

Medical College of Ohio
Opportunity Available 36A

Michael Reese Health Plan
Opportunity Available 36A

National Institutes of Health
Fellowship 19A

New England Medical Center
Opportunity Available 36A

North Suburban Clinic
Opportunity Available 37A

Nutrasweet Company, The
NutraSweet 6A

Permanente Medical Group, Inc., The
Opportunity Available 37A

Radiology Postgraduate Education
Book 21A

Ortho Pharmaceutical Corporation
All-Flex 8A
Ortho-Novum 7/7/7 ..... Third Cover, Fourth Cover

Osler Institute
Review Course 40A

St. Francis Regional Medical Center
Opportunity Available 37A

Syntex Laboratories, Inc.
AccuPoint 10A
Femstat 29A,30A,31A,32A
Tri-Norinyl ..... Second Cover,1A,2A

Thomas, Charles C., Publisher
Book 19A

Truman Medical Center, East
Opportunity Available 21A

Upjohn Company, The
Provera 3A,4A

Wright State University School of Medicine
Opportunity Available 21A

While every precaution is taken to insure accuracy, we cannot guarantee against the possibility of an occasional change or omission in the preparation of this index.

OBSTETRICS AND GYNECOLOGY

honoring Robert Scully, M.D.

*Any Six Days — December 1-9, 1987 — Ch

Now, your only BOARD REVIEW just before Oral
is also an excellent update for surgeons and preview for Writ

OBJECTIVES
- Increase basic knowledge in obstetrics and gynecology
- Improve clinical skills in diagnosis and treatment
- Assist residents and fellows in organizing study
- Prepare board candidates to pass board examinations
- Provide practicing surgeons with a review and update

METHODS
- HOME STUDY MATERIALS with question based on previous examinations
- SEMINAR with projection slides and syllabus
- LABORATORY with microscopic slides
- PRACTICE EXAMS with oral and written parts

* Dec. 1-6 (live); Dec. 7-9 (Video replay of Dec. 1-3). Any consecutive six days will complete program.

*"The faculty was outstanding. The most pleasant thing was learning a tremendous amount, not only from world-famous authorities but from people who are relatively unknown as well."**

PATHOLOGY
- Vulva
- Vagina
- Cervix
- Endometrium
- Myometrium
- Ovary
- Placenta

OBSTETRICS
- Pregnancy
- Labor
- Delivery
- Puerperium
- Abortion
- Fetal Development
- Neonatology

BASIC SCIENCES
- Anatomy
- Embryology
- Genetics
- Physiology
- Pharmacology
- Biochemistry
- Legal Problems

GYNECOLOGY
- Pelvic Relaxation
- Menstruation
- Contraception
- Infertility
- Endometriosis
- Sexuality & Rape
- Pediatric Gynecology

CLINICAL SCIENCES
- Anesthesiology
- Clinical Laboratory
- Endocrinology
- Infectious Disease
- Psychology
- Radiology
- Radiotherapy

ONCOLOGY
- Epidemiology
- Chemotherapy
- Vulva & Vagina
- Cervix
- Uterus
- Ovary
- Trophoblast

***Limited Enrollment*: OB—GYN REVIEW REGISTRATION**

Name _____

Address _____

City/State/Zip _____

Phone _____
(home) (office)

Mail today to: OB-GYN Review Course
1094 Dawn Lane
Terre Haute, IN 47802

☐ Check enclosed for $_____

*"Accommodations were comfortable..."**

LOCATION: The review course will be the Chicago area immediately before the boa in order to save you travel time and expe meeting will be held at the best combin luxury accommodations and bargain rates. perience proves that patient negotiation g best value for our participants. Our previ grams at Hyatts have been at or below $6 and $84 double. Please wait for instruction buying travel tickets.

*"...and those little extras..."**

LOWEST AIR FARES AND HOTI one-call travel and hotel reservations ca 232-0345 or toll-free (800) 538-6342 and yourself as a participant in the OB-GYN Course. We have negotiated group discount with American Airlines **below super saver rat**

*"...the most education for the money."**

FEES AND C.M.E. CREDITS:
- C.M.E. Credit: 60 hours
- Residents and Fellows: $420
- Practicing Surgeons: $630
- Repeating course first time within 3 years:
- Repeating course other times within 3 yea
- Add 10% to any payment after October 3
- Attendees not staying in course hotel adc
- Fees include no room or meals.
- $50.00 will reserve your position.
- Most home study materials will be mai half of registration fee is received.
- Additional materials will be sent after co of home study assignment.

*"The home study material was extremely to me."**

CANCELLATIONS: Refunds, subjec charge, will be made until the seminar begins
- Cancellations after mailing most hom material will require retention of ½ of the fee

*"...the course was remarkably compl pleasant."**

INFORMATION:

Joseph H. Selliken, Jr., M.D.
OBSTETRICS & GYNECOLOGY RE
1094 Dawn Lane, Terre Haute, Indiana
(812) 299-5658

**Comments by participants of our previous*

21-and 28-day regimens

ORTHO-NOVUM 7/7/7

Tablets

ethindrone/ethinyl estradiol TRADEMARK

ite tablet contains 0.5 mg norethindrone and 0.035 mg ethinyl estradiol. Each light peach tablet contains 0.75 mg drone and 0.035 mg ethinyl estradiol. Each peach tablet contains 1 mg norethindrone and 0.035 mg ethinyl ol. Each green tablet in 28-day regimen contains inert ingredients

TANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the and therefore should not be used as the basis for prescribing the product. This summary was prepared by from the complete prescribing information certain text, tables, and references. The physician should be hly familiar with the complete prescribing information before prescribing the product.

TIONS AND USAGE: PREVENTION OF PREGNANCY.

ELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES: Two studies have shown a positive tion between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive prescribed for any given patient should be that product which contains the least amount of estrogen that is ble with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral ptives be started on preparations containing .05 mg or less of estrogen.

INDICATIONS: Oral contraceptives should not be used in women with any of the following conditions: 1. ophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic s. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or ed estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Oral contraceptive tablets may ital harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who nant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard etus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of ora ptives or other estrogen-containing products.

WARNINGS

ette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk ases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 s of age. Women who use oral contraceptives should be strongly advised not to smoke.
use of oral contraceptives is associated with increased risk of several serious conditions including mboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practi rs prescribing oral contraceptives should be familiar with the following information relating to these risks.

MBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. An increased risk of thromboembolic and botic disease associated with the use of oral contraceptives is well established. Four principal studies in Great nd three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism ke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times ely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary m or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age.

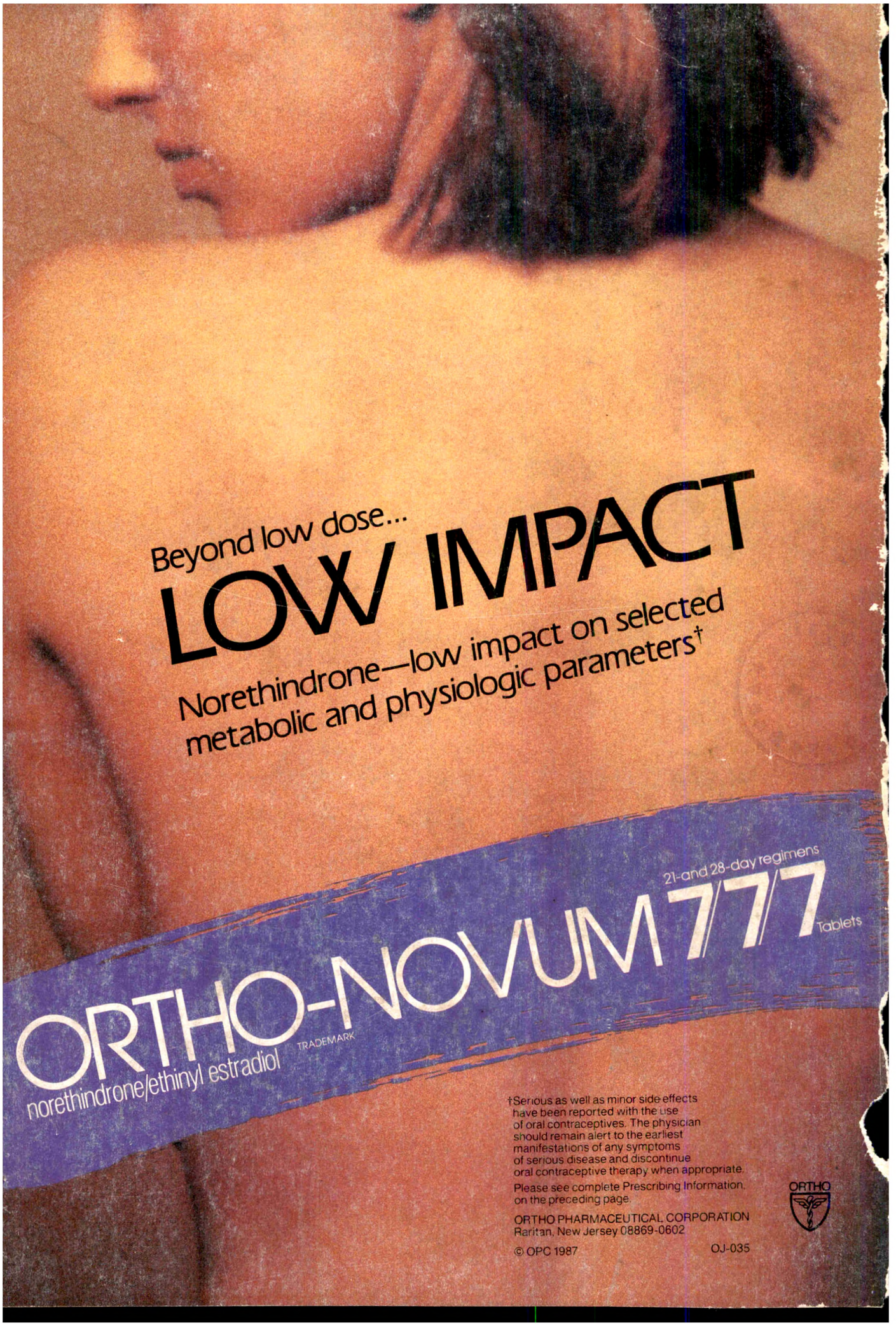
OVASCULAR DISORDERS: In a collaborative American study of cerebrovascular disorders in women with and predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in s and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study ed in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study ed in the United States found that past use of oral contraceptives was associated with increased risk of nhoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk related to duration of oral contraceptive use. **MYOCARDIAL INFARCTION:** An increased risk of myocardial n associated with the use of oral contraceptives has been reported confirming a previously suspected ion. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of ng risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether ent was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive as estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers out a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold d risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor mining the importance of these relative risks, however, the baseline rates for various age groups must be given consideration. The importance of other predisposing conditions mentioned above in determining relative and risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a xtent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists g discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the

Risk of Dose: In an analysis of data derived from several national adverse reaction reporting systems, British tors concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher romboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful ological studies to determine the degree of thromboembolic risk associated with progestogen-only oral ptives have not been performed. Cases of thromboembolic disease have been reported in women using these s, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic s, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an dent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large tive study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was d to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000); the risk ncentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous ntermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for ore years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with us use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is ued. Another study published at the same time confirms a previously reported increase of mortality in pill users diovascular disease. The study concluded that the mortality associated with all methods of birth control is low w that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The en for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 s a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50

The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up bortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolesity, diabetes, or history of preeclamptic toxemia and especially by cigarette smoking. The risk of myocardial n oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic s (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal sis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued tely. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral ptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type ed with an increased risk of thromboembolism or prolonged immobilization. 2. **OCULAR LESIONS.** There have orts of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral ptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete sion; onset of proptosis or diplopia; papilledema; or retinal vascular lesions and institute appropriate diagnostic ipeutic measures. 3. **CARCINOMA.** Long-term continuous administration of either natural or synthetic estrogen animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic gens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of rial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One n reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the rium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors ocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given; c ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no arketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of onal combination or progestogen-only oral contraceptives. Several studies have found no increases in breast women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive th documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral ptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of sed risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral ptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, te diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular ey elect to use oral contraceptives instead of other methods of contraception. 4. **HEPATIC TUMORS.** Benign denomas have been found to be associated with the use of oral contraceptives. One study showed that oral otive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. Cases of hepatocellular carcinoma, although rare, have been reported in women taking oral contraceptives. Retrospective studies conducted in the United States and Britain report an increased risk of hepatocellular carcinoma in oral contraceptive users, particularly with increased duration of use. 5. **USE IN OR IMMEDIATELY PRECEDING PREGNANCY. BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING.** The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7 fold increase in risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. **GALLBLADDER DISEASE.** Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. **CARBOHYDRATE AND LIPID METABOLIC EFFECTS.** A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. **ELEVATED BLOOD PRESSURE.** An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. **HEADACHE.** The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. **BLEEDING IRREGULARITIES.** Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. **ECTOPIC PREGNANCY.** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. **BREAST FEEDING.** Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS. General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. **INFORMATION FOR THE PATIENT: (See Patient Package Insert). DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **DRUG/LABORATORY TEST INTERACTIONS:** The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS and WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS). Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

ORTHO PHARMACEUTICAL CORPORATION

ORTHO


Beyond low dose...
LOW IMPACT
Norethindrone—low impact on selected metabolic and physiologic parameters†
21- and 28-day regimens
ORTHO-NOVUM 7/7/7 Tablets
TRADEMARK
norethindrone/ethinyl estradiol
†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.
Please see complete Prescribing Information on the preceding page.
ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869-0602
© OPC 1987
OJ-035
ORTHO